1 Microarray studies on a population based breast cancer cohort - search for gene profiles with prognostic and predictive value.

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Five hundred and twenty-four patients were operated for breast cancer during 1994 through 1996 at Karolinska Hospital, from 303 we had frozen tumor tissue, 6 patients actively denied participation in the study and 7 patients had migrated abroad. From these remaining, RNA was purified using the Quigen™ kit and quality and amount were controlled on agilent gels. Affymetrix™ based microarrays have been run and are presently running on samples with sufficient amount and RNA quality for 12 625 genes. The use of Proteinase K markedly increased the yield and quality of RNA.

Based on the 290 remaining patients, 18 have had a previous primary breast cancer before the study period, 15 had a synchronous cancer and 6 patients developed a contra-lateral cancer during or after the study period. The patients were treated according to the regional health care program. Twenty-four patients received neo-adjuvant chemotherapy. CMF and/or goserelin and/or tamoxifen (interrupted in sequence with megestrolacetate) were in general used to pre- and postmenopausal patients, respectively, based on the predefined criteria. Local and loco-regional radiotherapy was given according to predefined criteria. Date for relapse and therapies for metastatic disease as well as outcome were recorded. These variables are and will be analyzed in relation to the array profiles.

Using array profiles from cell lines together with the first patient set we have identified gene profiles associated with chemotherapy resistance and sensitivity, respectively. We aim at the meeting to present profiles with prognostic and predictive potential.

2 Survival impact of HER-2/Neu, Cox-2, urokinase plasminogen activator (UPA), cytokeratin 17/5, and other markers with long-term outcome of early breast cancer. Report from the British Columbia Tissue Micro-Array Project (BCTMAP).

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Tumor samples are available from over 19,600 Stage I-III breast cancer patients treated according to evolving British Columbia guidelines from 1978-1990. A tissue mico-array (TMA) was constructed from 930 of these patients, all of whom participated in randomized or phase II studies. Outcome was defined as 20 year Breast Cancer specific Survival (BrCaSS) with events defined as Breast Ca death. Follow up was median 16 years (ranges 11 - 24). Multiple tumor markers were tested, and results correlated with 20 yr BrCaSS for Positive vs. Negative.

	20 yr BrCaSS		
Marker	Positive	Negative	p value
Her-2-IHC	34%	48%	<0.0001
Her-2 FISH	33%	48%	0.0004
Her-2 CISH*	32%	47%	0.0001
Cytokeratins 17/56 IHC	35%	47%	<0.002
UPA IHC	46%	52%	0.03
Cox-2 IHC	32%	45%	0.026

No difference in BrCaSS was found for aromatase, Integrin-linked kinase (ILK), IGF-1 and Topo-isomerase-2. The negative predictive value of IHC vs FISH and CISH vs FISH was 96 and 97% respectively. The positive predictive value of IHC vs FISH and CISH vs FISH was 84 and 84% respectively. All tests, with the exception of HER-2 FISH and CISH were done by IHC. Results of other markers (VEGF, ER / PgR, hypoxia markers, etc.), and an interactive multivariate analysis adjusting for conventional prognostic factors and for all above markers will be presented.

Conclusions: 1. The TMA is a technique which provides opportunity for rapid screening of multiple genetic markers 2. Expression of Her-2/Neu, UPA, Cox-2 and Cytokeratin 17/56 (but not of Aromatase, ILK, TOPO-II and IGF-1) is associated with inferior BrCaSS. 3. HER-2 determination by CISH provides comparable results to FISH, with significant cost saving.

* CISH= chromogenic in-situ hybridization.

3 Expression of metastasis-associated 1 (MTA1) is associated with breast cancer recurrence.

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Background: Identification of primary breast tumor-specific genetic events that estimate the likelihood of distant recurrence would be valuable tools for the management of this disease. We previously reported that markers D14S62 and D14S51 detect loss of heterozygosity (LOH) at significantly higher rates in node-negative relative to node-positive primary cancers (68% versus 24%; p= 0.001). We have since determined that, in addition to a presumptive tumor suppressor gene, these LOH events encompass a metastasis-related gene (metastasis associated 1, or MTA1). MTA1 has homology to the nuclear receptor co-repressor 1 (NCOR1) gene, and is a subunit of the nuclear remodeling and histone deacetylation (NURD) complex.

Materials and Methods: Due to extensive homology between MTA1 and other MTA family members (MTA1L1 and MTA2), we identified MTA1-specific epitopes and generated a polyclonal antibody. Western blot studies were used to characterize the sensitivity and specificity of MTA1-specific antibody. An immunohistochemical (IHC) assay was developed for our MTA1-specific antibody to test formalin-fixed, paraffin-embedded archival tissues.

Results: Western blots on total, nuclear and cytoplasmic fractions of breast cancer cell lines indicate MTA1 is confined to the cell nucleus. Additional western blot studies on node-positive breast tumor lysates (N= 314) showed that MTA1 levels correlate with breast cancer-related factors such as ER (p< 0.0001), NCOR (p= 0.0001), AIB1 (p< 0.0001), FKHR (p< 0.0001) and SAFB (p< 0.0001). IHC analyses of untreated patients (N= 186, 57 recurrences, >8 year median follow-up) and controls (N= 73) show the following: 1. Univariate analyses indicated that MTA1 levels are higher in breast tumors than in normal tissue controls (p< 0.0001); 2. Univariate analyses show that high MTA1 correlated strongly with ER (p< 0.0001) and PgR (p= 0.0001); 3. In multivariate analyses of 997 patients that included positive nodes, tumor size, PgR, ER, ploidy, S-phase fraction and age at diagnosis, and adjuvant therapy, high MTA1 was a significant predictor of disease recurrence.

Conclusion: IHC studies of primary breast tumors indicate our MTA1-specific antibody may serve as a prognostic marker of breast cancer recurrence.

4 Loss of expression of tight junction molecules in breast tumour tissue correlates with poor clinical outcome in patients with breast cancer.

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Background: Tight junctions are the topical most structures in both endothelial and epithelial cells. Interaction and penetration of endothelium by cancer cells is an important step in the formation of metastases, indicating that changes in tight junction function will be a key aspect. This study aimed to determine whether a correlation exists between levels of tight junction molecules in breast cancer primary tumours and patient outcome.

Methods: Breast cancer primary tumours (n=114) and matched background tissue (n=30) were processed for RNA extraction and frozen sections. RNA was reverse transcribed and quantified before analysis by quantitative-PCR. Amongst the tight junction molecules targeted were: ZO-1, JAM-1, Occludin and Claudins-1 and -5. The results were expressed as copy numbers of transcript/50 ng RNA (standardised by b-actin). Frozen sections from matched tissues (tumour and background) were immuno-stained with ZO-1, Occludin, Claudin-1 and Claudin-5 antibodies.

Results: The levels of transcripts of ZO-1, Occludin and JAM-1 were significantly lower in patients that had metastatic disease, compared with those that remained healthy, after a median follow-up of 72.2 months (ZO-1: 19.3 +/- 15 compared to healthy 273 +/- 81.5, p=0.003; Occludin: 93.1 +/- 31.7 compared to healthy 331.3 +/- 98.4, p=0.02; JAM-1: 5.18 +/- 2.11 compared to healthy 56.4 +/- 20.1, p=0.01). JAM-1 was also significantly lower in patients that had local recurrences or had died of breast cancer, when compared to those that remained healthy (local recurrences 8.32 +/- 2.22, p=0.02; died of breast cancer 20.6 +/- 8.46, p=0.05). Immunohistochemistry confirmed these results, as there were decreased levels in staining for Occludin and ZO-1. Moreover, there was a distinct difference in distribution of staining for ZO-1, Occludin and Claudin-1 and -5. In normal tissue, staining was confined to the intercellular regions whereas in the tumour tissues the staining was diffuse and cytosolic.

Conclusion: We conclude that low levels of tight junction molecules in breast cancer are associated with poor prognosis in patients. Regulation of tight junctions could be of fundamental importance in the prevention of metastasis of breast cancer cells.

5 FHIT inactivation and its prognostic significance in breast cancer.

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Background: The fragile histidine triad (FHIT) gene, which is frequently lost in many cancers, was identified as a candidate tumor suppressor gene at chromosome 3p locus 14.2. The mechanism of FHIT suppression remains largely unknown. Little information is available on the relationship between Fhit expression and prognosis in breast cancer.

Materials and Methods: To confirm the tumorigenic role of FHIT, sporadic invasive breast cancers were tested for the "two hits" required to inactivate this gene. Microsatellite loss of heterozygosity (LOH) was considered as the first hit. To examine the possibility that hypermethylation serves as the second hit for FHIT inactivation, we analyzed methylation of 5'-CpG of FHIT by methylation specific PCR. To determine whether Fhit protein loss correlates with other established pathological-clinical parameters or prognosis, we assessed Fhit expression using immunohistochemistry in 166 invasive breast carcinomas.

Results: The tumors showing both LOH and hypermethylation showed complete loss of FHIT protein expression. In addition, a significant positive association was found between the existence of LOH and hypermethylation ($p=0.04$). Lost or significantly decreased Fhit protein expression was identified in 70 cases (42.2%). Fhit expression was inversely correlated with histological grade ($p < 0.0001$), negative estrogen receptor status ($p = 0.0016$), p53 overexpression ($p = 0.004$), and tumor proliferation activity ($p = 0.0006$). Furthermore, aberrant Fhit expression was associated with worse prognosis ($p= 0.0086$, by log-rank test).

Discussion: To our knowledge, this study provides the first evidence that biallelic inactivation of the FHIT by LOH and hypermethylation leads to the complete inactivation of FHIT gene in patients with breast cancer. Silencing of the FHIT gene is associated with higher malignant phenotypes and appears to be a prognostic factor in breast cancer. "Demethylating" may be a potential method for breast cancer prevention and treatment.

6 Molecular mechanism of estrogen receptor sequesteration in cytoplasm.

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We report the identification of a metastatic-associated protein 1 (MTA1), a component of histone deacetylase and nucleosome remodeling complexes, is a potent corepressor of ERE transcription as it blocked the ability of estradiol to stimulate the estrogen receptor-mediated transcription. Histone deacetylase inhibitor trichostatin A blocked the MTA1-mediated repression of ERE transcription. Overexpression of MTA1 in breast cancer cells was accompanied by a significant enhancement in the ability of cells to invade, and grow in an anchorage-independent manner. In addition to MTA1, recently we have identified a naturally occurring variant of MTA1 that contains a novel sequence of 33 amino acids. Alternative splicing in the MTA1 gene by a cryptic splice site generates this variant, a short form MTA1 (MTA1s). MTA1s localizes in the cytoplasm, sequesters ER in the cytoplasm, directly interacts with ER, blocks ER target gene expression, but enhances non-genomic responses of ER. Deletion of the defined motif in MTA1s abolishes its corepressor function and interaction with the ER, and restores nuclear localization of ER. MTA1s expression in breast cancer cells prevents ligand-induced nuclear translocation of ER, and stimulates anchorage-independent growth and tumorigenicity of cells in vivo. malignant phenotypes. MTA1s expression is elevated in human breast tumors with no nuclear ER. The regulation of the cellular localization of ER by MTA1s represents a novel mechanism for redirecting nuclear receptor signaling by nuclear exclusion. In addition, estrogen responsiveness of breast cancer cells may be influenced by the ratio of the wild-type- and variant- MTA1, and the pathways that infunences its functions.

7 Enhanced benefit from adjuvant chemotherapy in breast cancer patients classified high-risk according to uPA and PAI-1 (n=3424).

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Risk assessment and prediction of therapy response are prerequisites for individualized adjuvant therapy decisions in primary breast cancer. Current guidelines define a subgroup (e.g., receptor positive) with a low number of affected lymph nodes for which adjuvant endocrine therapy is recommended, but adjuvant chemotherapy is only optional. This paper considers whether relapses which would occur using only conventional factors for therapy decisions could be avoided or delayed by measuring invasion factors uPA and PAI-1 and by administering chemotherapy to the high-risk group.

Strong evidence for a predictive impact of the combination of uPA and PAI-1 on response to chemotherapy was found in an analysis of 3424 primary breast cancer patients from Munich and Rotterdam. uPA and PAI-1 levels were measured by ELISA in tumor tissue extracts, and a binary variable uPA/PAI-1 (either high vs. both low) was used as previously validated. After median follow-up of 83 months, uPA/PAI-1 has a significant impact on DFS in Cox multivariate analysis [$P < 0.001$; HR= 2.0 (1.8-2.3)]. The key observation is that patients with high uPA/PAI-1 have an enhanced benefit from adjuvant chemotherapy compared to those with low levels. This effect is seen as a significant interaction between chemotherapy and uPA/PAI-1 for the entire collective [$P < 0.003$; HR: 0.68 (0.53 - 0.88)] and separately within nodal subgroups (0-3, > 4 involved nodes). This enhanced benefit of chemotherapy in high uPA/PAI-1 patients occurs over and above the significant benefit from both adjuvant therapies (chemo-, endocrine therapy) in all patients.

Thus, uPA/PAI-1 are not just significant prognostic but also significant predictive factors and are essential for supporting individualized therapy decisions. Our results should not be interpreted as evidence for removing endocrine therapy where indicated, but rather for measuring uPA and PAI-1 and — in light of the known additive benefits of endocrine and chemotherapy — for administering both adjuvant endocrine therapy and adjuvant chemotherapy to the high-risk group.

8 The use of predicting factors and surrogate markers in breast cancer biopsies treated with targeted erbB tyrosine kinase inhibitor.

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Background: Over-expression of erbB receptors is associated with aggressive breast cancers. Therapeutic strategies targeting these oncoproteins are in clinical trials. One approach is the use of a monoclonal antibody to erbB2, Herceptin. Studies performed in vitro have attributed the therapeutic potential of Herceptin to enhance intracellular degradation resulting in a functional inhibition of erbB2. Another effective approach is the use of tyrosine kinase inhibitors (TKIs) that block the nucleotide-binding site of the erbB kinases, specifically erbB1 and erbB2. An alternative way to enhance degradation and inhibit activity of erbB proteins involves targeting the heat shock protein 90 (Hsp90) using benzoquinone ansamycins such as geldanamycin (GA). Hsp90 forms complexes with erbB2 proteins and stabilizes them. GA blocks ATP binding to Hsp90 resulting in poly-ubiquitination and destruction of the erbB2. In contrast, the TKI group of drugs is highly selective to erbB receptors blocking only the nucleotide-binding site of tyrosine kinase proteins. Consequent to blocking kinase activity, most downstream signaling pathways are inhibited leading to growth arrest. In this work, we used cancer tissue biopsies from patients before and after TKI treatment to understand the mechanism and the factors associated with response or non-response to TKI treatment.

Materials and Methods: Breast cancer biopsies from patients, before and after TKI treatment, were immunostained for erbB1 and erbB2. Their phosphorylated forms and phosphorylated ERK (pERK) (a downstream signal) were used as a surrogate marker of response (antibodies were purchased from Cell Signaling and Ventana). Levels of staining were quantitated by microscope based image analysis.

Results: Patients with high levels of EGFR, HER-2 and pERK responded to TKI. Their response was confirmed by using surrogate biomarkers as well as objective clinical response. The surrogate biomarker pERK showed dramatic downregulation in patients that responded to TKI therapy.

Discussion: Using biomarkers to predict and monitor response to TKIs can help stratify best patient populations for TKI treatment. Strategies combining the effectiveness of chaperone-mediated degradation with the selectivity of TKIs hold promise for breast cancer therapy.

9 Successful quality assurance program for HER2 testing in the NSABP trial for Herceptin.

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Background: The NSABP is conducting an adjuvant clinical trial (B-31) in which patients with node-positive, HER2 overexpressing breast cancer are randomized to Adriamycin, cyclophosphamide followed by Taxol (AC→ T) vs. Adriamycin, cyclophosphamide followed by Taxol plus trastuzumab (AC→ TH). When the trial was initiated, eligibility was determined by HER2 testing based on results from local laboratories, i.e., 3+ immunohistochemistry (IHC) or gene amplification by FISH. Centralized re-testing of the first 104 cases entered into B-31 based on IHC demonstrated poor reproducibility, i.e., about 20% could not be confirmed as positive when IHC is performed by smaller volume laboratories (Paik S et al, J Natl Cancer Inst 94:852-4, 2002). Based on this, the eligibility criteria in Protocol B-31 were amended to require HER2 testing from NSABP-approved laboratories for patient entry. Lab approval was based on volume of testing and demonstration of high concordance between IHC and FISH. 22 laboratories in the US have been approved to date. The quality control of HER2 testing after the amendment is now presented. Methods: Central PathVysion® FISH assay was performed on 100 cases entered since the amendment. Results: Definitive gene amplification could be demonstrated in 95 of 100 cases on tissue microarray. Five equivocal cases await confirmatory testing on individual sections. Conclusion: Our data suggest improved quality assurance when use of high-volume laboratories is required for HER2 testing. Detailed comparison of testing with IHC vs. FISH will be presented at the meeting.

10 Meta-analysis of the relationship between serum trastuzumab concentrations and shed HER2 extracellular domain (ECD).

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Background: The humanised MAb trastuzumab (T) targets the HER2 receptor ECD. Some patients with breast cancer have high circulating shed ECD levels. These may be associated with a poorer prognosis and could reduce the effectiveness of T.

Methods: Data for a total of 114 patients from 4 phase I/II studies of T who gave 391 samples for both ECD and trough T concentration were pooled. Three studies were in MBC (one using a weekly maintenance dose of 2mg/kg; two using a 3-weekly maintenance dose of 6mg/kg). The fourth study was in NSCLC and used the weekly regimen above. The homogeneity of log-ECD data among the studies was tested using ANOVA. Association between T concentration (dichotomised at 40µg/ml) and clinical response was tested by chi-square statistics; 40µg/ml was selected as the cut-off because it was approximately the average trough T concentration observed in partial responders. The homogeneity of clinical response among the studies was examined by comparing 95% CI for the log-odds ratios before the pooled log-odds ratio was calculated. A logistic regression model (SAS V8.02) was used to examine the predictive value of log-ECD for estimating clinical response. A random effect model was used to examine the relationship between log-ECD and trough T concentrations in the presence of study identifier as the random effect, in addition to age, weight and race as the fixed effects.

Results: T concentration was a highly significant (p=0.0001) predictor of clinical response: patients with T concentrations >40µg/ml had a 2.8-fold (95% CI: 1.6 to 4.7) greater chance of having a good clinical response (partial or complete).Clinical response and log-ECD were not significantly related, but log-ECD and trough T concentrations showed an inverse relationship (p=0.04).

Conclusions: Circulating ECD levels may not be a strong predictor of therapeutic response to T treatment due to the large variability (CV=300%) observed in ECD levels. Serum T concentrations may be a significant predictor of therapeutic response.

11 Chemoendocrine therapy for node-negative breast cancer: International Breast Cancer Study Group (IBCSG) trials VIII and IX.

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Between 1988 and 1998, the IBCSG conducted two randomized trials to evaluate adjuvant chemoendocrine therapies for patients with node-negative disease. All patients had a histologically proven unilateral breast cancer, stage T1a, T1b,T2a, T2 or T3, pN0, M0 (UICC 1987), with either ER-positive or ER-negative primaries. ER-unknown was only allowed if the ER determination was not possible due to lack of material. Steroid hormone receptor concentrations in the primary tumors were determined by standard methods and ER concentrations greater than 10 fmol per milligram of cytosol protein were considered positive; lower values, negative. Steroid hormone receptor determination by immunohistochemistry (IHC) was allowed later in the study; ER was determined by IHC for 32% in premenopausal and 29% in postmenopausal patients. Postmenopausal status was defined as having one of the following sets of characteristics: i) more than 52 years old with at least 1 year amenorrhea; ii) 52 years old or younger with at least 3 years amenorrhea; iii) 56 years old or older with hysterectomy but no bilateral oophorectomy; iv) biochemical evidence of cessation of ovarian function (for doubtful cases). All other patients were considered pre/perimenopausal. In IBCSG VIII, 1063 eligible pre/perimenopausal women were randomized to: (a) goserelin (3.6 mg s.c. monthly) x 24 months, (b) "classical" CMF (cyclophosphamide at 100 mg/m2 on days 1-14, orally; methotrexate at 40 mg/m2 on days 1 and 8, intravenously; and 5-fluorouracil at 600 mg/m2 on days 1 and 8, intravenously) x 6 courses, (c) "classical" CMF x 6 followed by goserelin x 18 months (total of 24 months of treatment). In IBCSG IX, 1669 eligible postmenopausal women were randomized to: (a) tamoxifen alone (20 mg/day, for 60 months), or (b) "classical" CMF followed by tamoxifen (20 mg/day, for 57 months)). The median follow-up was 5.7 years for VIII and 6 years for IX. Results for both trials demonstrated a profound difference in treatment effects according to ER status of the primary tumor. For ER-negative cohorts, chemotherapy containing regimens provided significant benefit compared with regimens of endocrine therapy alone. In contrast, for ER-positive cohorts, the chemotherapy did not add to the benefit already obtained by endocrine therapy alone, especially for patients who were 40 years of age or older. The contrast in treatment differences between ER-negative and ER-positive cohorts observed in these node-negative studies is much sharper than the contrast observed for studies including patients with node-positive disease. The results for IBCSG VIII and IX will be updated for the meeting. The results will be compared with those from trials in node-positive disease.

12 Changes in bone mineral density caused by anastrozole or tamoxifen in combination with goserelin (± zoledronate) as adjuvant treatment for hormone receptor-positive premenopausal breast cancer: results of a randomized multicenter trial.

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Background: Combined endocrine treatment using goserelin (GOS) and tamoxifen (TAM) has been shown to be as effective as or even superior to standard chemotherapy in the adjuvant treatment of premenopausal patients with hormone-responsive breast cancer (JCO, in press).

Patients and Methods: In order to further improve these results, ABCSG Trial 12 is currently being conducted in 1250 premenopausal patients with estrogen- and/or progesterone receptor-positive disease to test anastrozole (ANA) in combination with GOS. One major concern when using complete endocrine blockade in premenopausal patients is the unknown treatment effect on bone mineral density (BMD). Bisphosphonates may both inhibit detrimental effects of combination endocrine treatment and increase relapse-free survival. This 4-arm trial thus compares GOS (3.6mg q 28d po) + TAM (20mg/d po) with GOS + ANA (1mg/d po), both ± zoledronate (ZOL; 4mg/6m), respectively, for a total of 3 years. BMD measurements were performed by standardized densitometry. For this analysis, lumbar spine and trochanter femoris densitometry changes were entered in a regression model as time-dependent. Changes over time were normalized to interindividual differences of baseline investigations.

Results: 231 women out of a total of 667 patients have so far been subjected to BMD measurements. At least 2 measurements are available for 138 patients. Median age at diagnosis is 44.8 years (range: 26-55). After 6 months of treatment, the cohorts receiving ZOL had significantly better lumbar spine BMD (p>0.0001). Comparing the ANA and TAM treatment groups, patients given ANA suffered more advanced deterioration than those receiving TAM (p=0.0125). In multivariate analysis, interaction was shown to be significant between ZOL and treatment effects.

Conclusions: This preliminary analysis confirms that ZOL is able to counteract BMD deteriorations in premenopausal patients with hormone receptor-positive breast cancers treated with complete endocrine treatment with GOS and TAM or ANA. Without the bisphosphonate, BMD deterioration is more pronounced in patients receiving GOS + ANA than those receiving GOS + TAM. Longer-term BMD monitoring will be necessary to determine whether these effects are prolonged.

13 A randomized trial of goserelin (Zoladex™) + tamoxifen versus goserelin + anastrozole (Arimidex™) in pre/perimenopausal patients with hormone dependent advanced breast cancer.

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Rationale: Gonadotropin-releasing hormone (GnRH) agonists (as ovarian ablative therapy), in particular goserelin (GOS), are used for the treatment of breast cancer in women with functioning ovaries. The addition of tamoxifen (TAM) to GOS results in significant efficacy benefits in treatment of advanced breast cancer (ABC) (Klijn et al. *J Clin Oncol* 2001; **19**: 343-353). Given that we have previously shown the aromatase inhibitor, anastrozole (AN), to be highly effective for the treatment of hormone-dependent ABC (Milla-Santos et al. *Breast Cancer Res Treat* 2000; **64[1**]: abstract 173), we implemented a further study in January 1999 to compare GOS + TAM (GOS/TAM) vs GOS + AN (GOS/AN) in a group of pre/perimenopausal patients (pts) with hormone-dependent ABC. Patients and Methods: Prior to randomization, pts had to fulfil the following requirements: 1. Histopathologic diagnosis of ABC; 2. Measurable/evaluable lesions; 3. Eastern Cooperative Oncology Group (ECOG) Performance Status 0-2; 4. Life expectancy >4 months; 5. No previous hormonal therapy for advanced disease; 6. No previous hormonal adjuvant therapy; 7. Pre/perimenopausal status; 8. Estrogen receptor positive (ER+) tumor; 9. Adequate organ functions; and 10. Signed informed consent. Patients received either GOS/TAM: GOS 3.6 mg/depot injection every 28 days + TAM 20 mg once daily (od), orally (po), or GOS/AN: GOS 3.6 mg/depot injection every 28 days + AN 1 mg od po. Results were evaluated 3 months after therapy was started by means of WHO guidelines for measurable lesions and ECOG criteria for non-measurable but evaluable bone lytic lesions. Safety was evaluated by means of WHO criteria. The proportion of pts with Overall Response (OR = Complete Response [CR] + Partial Response [PR]) and Clinical Benefit (CB = CR + PR + Stable Disease) was compared by logistic regression. Results: From January 1999 to December 2001 a total of 119 pts (mean age 45 years) were included in the trial; 58 received GOS/TAM and 61 received GOS/AN. Both groups of pts were well balanced with respect to demographics and disease characteristics. OR rate was 53% for the GOS/TAM vs 80% for the GOS/AN (odds ratio 0.281; confidence limit [CL] 0.124-0.635; p=0.0023). CB was greater for pts receiving GOS/AN (p=0.0506). Median duration of CB was 8.3 months vs 12.1 months for GOS/TAM and GOS/AN, respectively. Patients on GOS/AN survived significantly longer; median time to death was 14.3 months vs 18.9 months for GOS/TAM and GOS/AN, respectively (hazard ratio 0.413; CL 0.279-0.611; p=0.0001). The incidence of side-effects was low in both groups of pts. Conclusions: Our data suggest GOS/AN is an efficient and well-tolerated treatment, and should be considered for first-line therapy in pre/perimenopausal women with hormone-dependent ABC.

14 Predictive factors and response to letrozole vs. tamoxifen.

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Background: CA 15-3 and CEA are traditional tumor markers whereas the HER-2/neu oncoprotein not only reflects tumor burden but also tumor aggressiveness. We analyzed the effect of CEA, CA-15-3 and the oncoprotein HER-2/neu on response to 1st line treatment of metastatic breast cancer patients with letrozole vs. tamoxifen.

Materials and Methods: Pretreatment sera were obtained from the pivotal study which showed that letrozole was superior to tamoxifen for first-line treatment of metastatic breast cancer (JCO 2001; 19:2596-606). Retrospectively, pretreatment serum samples (548/907 patients) were analyzed for CEA, CA 15-3, and HER-2/neu (Bayer Immuno 1 automated assay).

Results: Objective response rate (ORR)(CR+PR) was significantly lower for patients with elevated serum HER-2/neu (15%) than for patients with normal serum HER-2/neu (32%) (odds ratio=2.74; P<0.0001). Clinical benefit rate (CBR)(CR+PR+NC³24 weeks) was similarly lower (elevated 30%, normal 51%; odds ratio=2.40; P<0.0001). Time to progression (TTP) was shorter for patients with elevated serum HER-2/neu (median 5.7 months (mos) vs 9.5 mos; hazard ratio 0.56; P<0.0001). In patients who had normal serum HER-2/neu, ORR (39% vs. 27%, P=0.01), CBR (56% vs. 45%, P=0.03) and TTP (median 12.2 vs. 8.5 mos, P=0.002) were significantly better in patients who received letrozole than tamoxifen. ORR (21%) and CBR (37%) were lower in patients with an elevated serum CEA level than in patients with normal serum CEA (ORR 32%, CBR 51%; ORR P=0.004; CBR P=0.001). Median TTP (6.0 mos) was also significantly shorter for these patients (median 9.5 mos for patients with normal CEA; P<0.0001). In patients with elevated CEA, letrozole was significantly superior to tamoxifen in ORR (27% vs 16%, P=0.04), CBR (45% vs 29%, P=0.01) and TTP (median 8.2 mos vs 3.5 mos, P=0.008) for patients treated with letrozole and tamoxifen, respectively. In multivariate analysis, elevated serum HER-2/neu predicted decreased ORR and CBR, and shorter TTP; and letrozole demonstrated a significantly higher ORR and CBR, and longer TTP than tamoxifen.

Conclusion: Letrozole demonstrated a significantly higher ORR and CBR, and longer TTP than tamoxifen when adjusted for tumor burden and the HER-2/neu oncoprotein.

15 Superiority of dose-dense (DD) over conventional scheduling (CS) and equivalence of sequential (SC) *vs.* combination adjuvant chemotherapy (CC) for node-positive breast cancer (CALGB 9741, INT C9741).

Citron M, Berry D, Cirrincione C, Carpenter J, Hudis C, Gradishar W, Davidson N, Ingle J, Martino S, Livingston R, Winer E, Muss H, Norton L.

Mathematical modeling has suggested an advantage of DD chemotherapy (shortened inter-treatment intervals), and no detriment for SC *vs.* CC in certain clinical settings. To test these hypotheses we conducted a 2x2 factorial clinical trial as follows:

Arm 1 (CS,SC): Ax4→ Tx4→ Cx4 q 3 weeks
Arm 2 (DD,SC): Ax4→ Tx4→ Cx4 q 2 weeks with filgrastim
Arm 3 (CS,CC): ACx4→ Tx4 q 3 weeks
Arm 4 (DD,CC): ACx4→ Tx4 q 2 weeks with filgrastim

A=doxorubicin 60mg/m2, T=paclitaxel 175 mg/m2 over 3 hours, and C= cyclophosphamide 600mg/m2. Tamoxifen 20 mg was given post-chemotherapy. Between 9/97 and 3/99 2005 patients were accrued. Median age was 50 yrs old. Median number of positive nodes was 3; ten or more nodes were present in 12%. 67%. were ER+.

Results: No neutropenic deaths were reported. Less grade 4 neutropenia was encountered in the DD arms. 13% in arm 4 required RBC transfusion compared to <4% on other arms (p=0.0002). The protocol was designed for analysis 3 yrs after closure. The primary endpoint, disease-free survival (DFS), was superior for DD over CS (RR=0.74, p= 0.0072), as was overall survival (OS) (RR=0.69, p=0.014). The 3-year DFS was 85% for DD and 81% for CS; the 3-year OS was 92% for DD and 90% CS. There was no difference in either DFS or OS between SC vs. CC. With median follow-up of 36 months 315 pts had relapsed or died without relapse *vs.* 515 expected failures under the null hypothesis.

Conclusion: Because the number of events is lower than expected, statistical comparisons must be considered preliminary. However, to date, DD is superior in efficacy with less severe neutropenia. SC given as DD retains efficacy with less anemia.

16 Findings from two decades of National Surgical Adjuvant Breast and Bowel Project clinical trials involving breast cancer patients with negative axillary nodes.

Fisher B, Jeong J-H, Bryant J, Mamounas EP, Dignam JJ, Wolmark N. (NSABP), Pittsburgh, PA

Since 1981, 6 NSABP trials involving 11,699 breast cancer patients with negative axillary nodes have provided sound evidence that 1) chemotherapy (chemo) benefits those with ER-negative tumors, 2) tamoxifen (TAM) alone improves the outcome of those with ER-positive tumors, 3) chemo plus TAM is superior to TAM alone in those with ER-positive tumors, 4) the administration of TAM with chemo for those with ER-negative tumors adds no benefit, and 5) radiotherapy with TAM is superior to either alone for patients with tumors ≤ 1 cm in largest diameter. Since the duration of follow-up is between 10 and 16 years in all but one of these studies, meaningful updates of primary findings are available. In addition, the long-term follow-up permits the analysis of cause specific mortality to assess the impact of these treatments on breast cancer deaths in patients with negative axillary nodes. Finally, issues concerning whether there are subgroups of patients who obtain more (or less) benefit from adjuvant therapy can now be more adequately addressed as well as those related to contralateral breast cancer. The findings will be considered relative to current treatment strategies for node-negative patients.

17 A randomized trial comparing CEF to CMF in premenopausal women with node positive breast cancer: update of NCIC CTG MA.5.

Levine MN, Pritchard KI, Bramwell VHC, Shepherd LE, Tu D, Paul N. McMaster University, Hamilton, ON, Canada; University of Toronto, Toronto, ON, Canada; University of Western Ontario, London, ON, Canada; Queens' University, Kingston, ON, Canada

Certain anthracycline-containing adjuvant chemotherapy regimens are associated with improved disease-free survival (DFS) and overall survival (OS) compared to classic CMF in women with early stage breast cancer. Between 1989 and 1992, 710 pre- and perimenopausal women with axillary node positive breast cancer were randomized to CEF (cyclophosphamide 75 mg/m^2 orally Days 1-14, epirubicin 60 mg/m^2 IV Days 1 & 8, and 5FU 500 mg/m^2 IV Days 1 & 8) or CMF (cyclophosphamide 100 mg/m^2 orally Days 1-14, methotrexate 40 mg/m^2 IV Days 1 & 8, and 5FU 600 mg/m^2 IV Days 1 & 8). Based on follow-up to May 1997 (median follow-up 59 months), there was a statistically significant improvement in DFS and OS for CEF compared to CMF (J Clin Oncol 1998; 16, 2651). The trial results are now updated with a median follow-up of 106 months. The 10-year DFS is 52% for patients who received CEF compared to 45% for CMF patients, hazard ratio CMF versus CEF, 1.31 (stratified Wilcoxon test P=0.005). The corresponding data for 10-year OS are 62% and 58% respectively, hazard ratio CMF versus CEF, 1.18 (P=0.047). The rates of acute leukemia are unchanged since the original report while the rates of congestive heart failure are slightly higher but acceptable; 4 cases (1.1%) in the CEF group versus 1 (0.3%) in the CMF group. In conclusion, in the MA.5 trial, the previously demonstrated benefit of CEF over CMF adjuvant chemotherapy is maintained with longer follow-up.

18 Targeting the epidermal growth factor receptor pathway improves the anti-tumor effect of tamoxifen and delays acquired resistance in a xenograft model of breast cancer.

Massarweh S, Shou J, DiPietro M, Mohsin SK, Hilsenbeck SG, Wakeling AE, Brown PH, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; Astra-Zeneca, Macclesfield, United Kingdom

The antiestrogen tamoxifen is the most widely used endocrine therapy for breast cancer. However, both de novo and acquired resistance are major problems, and novel therapies to overcome resistance are needed. We have shown that tamoxifen stimulates growth of HER2 overexpressing breast tumors as a mechanism of de novo resistance, and that inhibition of the HER2 pathway using ZD1839 ('Iressa') restores tamoxifen sensitivity in these tumors. In non-HER2 overexpressing breast cancer, we have shown in our xenograft model that acquired resistance to tamoxifen is caused by tamoxifen-stimulated growth. Data from in vitro studies have shown that emergence of tamoxifen resistance is associated with epidermal growth factor receptor (EGFR) overexpression. To examine whether inhibiting the EGFR pathway can modulate endocrine response in non-HER2 overexpressing breast cancer, MCF-7 tumors were established in nude mice in the presence of exogenous estrogen. Mice bearing established tumors then received either continued estrogen supplementation, tamoxifen or estrogen deprivation, with or without ZD1839. In mice receiving estrogen, ZD1839 had no effect on tumor growth. In contrast, ZD1839 improved the antitumor effect of tamoxifen, and markedly delayed the emergence of acquired resistance from 2-3 months to over 6 months. When ZD1839 was added to tamoxifen-sensitive tumors later in the treatment course, delay in acquired resistance was modest, suggesting that initial combined tamoxifen and ZD1839 is a more effective treatment strategy. In mice treated with estrogen deprivation, there was no demonstrable benefit from adding ZD1839 in improving response or delaying resistance. These studies support the concept that activation of the EGFR pathway enhances tamoxifen's agonist effect and contributes to acquired resistance, and show that eliminating this agonist effect can improve initial response and delay acquired resistance. Data from this model provides evidence that combined ER-targeted therapy, especially tamoxifen, with growth factor pathway inhibitors, like ZD1839, may be a highly effective treatment for ER-positive breast cancer and should be tested in the clinical setting.
('Iressa') is a trademark of the AstraZeneca group of companies.

19 Prevention of breast cancer in MMTV-ErbB2 transgenic mice using the tyrosine kinase inhibitor, ZD1839 ('Iressa').

Lu C, Zhang Y, Hill J, Celestino J, Steinbis E, Bui D, Wu K, Kim H, Schiff R, Osborne K, Hilsenbeck S, Wakeling A, Brown P. Baylor College of Medicine, Houston, TX; Astra-Zeneca Pharmaceuticals

ZD1839 ('Iressa') is an orally active, selective epidermal growth factor receptor-tyrosine kinase inhibitor, which blocks signal transduction pathways in epithelial cells. We hypothesized that ZD1839 will block signal transduction, suppress the growth of breast cells, and prevent breast cancer development. We first measured the expression of phospho-MAPK in MCF7 cells after growth factor stimulation in the absence or presence of ZD1839. We found that ZD1839 suppressed the induction of phospho-MAPK expression. We then investigated the effect of ZD1839 on the growth of normal, premalignant, and malignant breast cells in vitro. Using proliferation assays we demonstrated that ZD1839 inhibits the growth of normal (184, HMEC) and immortalized (MCF10A, 184B5) human breast epithelial cells, as well as cancer cells(MDA MB 468). Next, we investigated the ability of ZD1839 to suppress mammary tumor formation in MMTV-c-erbB2 transgenic mice. We treated the mice with vehicle or with one of two doses of ZD1839 (10, 100, mg/kg) orally from 3 to 12 months. We observed that ZD1839 suppressed mammary tumor formation in these mice. Median time to tumor development in vehicle treated mice was approximately 230 days of age, while it has not been reached after 290 days of age in the mice treated with high dose ZD1839. After 200 days of treatment, 90% of vehicle treated mice developed mammary tumors, while only 15% of mice treated with high dose ZD1839 developed tumors. This was a statistically significant delay in time to tumor formation (P=0.0001). Chronic treatment with ZD1839 caused minimal toxicity. Drug treatment was well tolerated with no toxicity observed until 2-3 months of treatment; after that time some mice showed signs of cutaneous toxicity, and by 200 days approximately 50% of the high dose mice developed eye irritation and/or hair loss. We are now measuring the effect of ZD1839 on proliferation, apoptosis and downstream signals of peptide growth factors in the mammary gland tissue from these mice. These studies provide the preclinical foundation to develop tyrosine kinase inhibitors as cancer preventive agents in future human breast cancer prevention trials.
'Iressa' is a trademark of the AstraZeneca group of companies.

20 Open-label, phase II, multicenter trial of ZD1839 ('Iressa') in patients with advanced breast cancer.

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Epidermal growth factor receptor-tyrosine kinase (EGFR-TK) is recognized as a key modulator of tumor cell function and is considered to be a viable drug target in a variety of solid tumors, including breast cancer. The clinical benefit and safety of the oral, selective, EGFR-TK inhibitor ZD1839 ('Iressa') were evaluated in this nonrandomized, open-label, Phase II, multicenter study of patients with metastatic breast cancer. There were no entry limitations regarding the number of prior chemotherapy or hormonal regimens. Patients were treated with an oral, daily 500 mg dose of ZD1839 until disease progression, intolerable toxicity, or consent withdrawal. Dosage reduction to 250 mg daily was allowed for toxicity. The primary end point was the clinical benefit rate (complete response + partial response [modified ICC/WHO criteria] + stable disease) at 6 months. The patient accrual goal of 63 patients (ages 34.9-80 y) was met on April 17, 2002. Demographic characteristics are summarized below. Details of efficacy and safety results will be presented.

Median age, y	52.9	HER2 status, n +/-/unknown	28/32/3
Race, n	53/6/2/2	Hormone receptor status,	28/34/1
white/black/Hispanic/other		n +/-/unknown	
Metastatic disease site, n		Prior treatment, n	
Bone only	2	Taxane	45
Soft tissue only	6	Anthracycline	32
Visceral	51	Taxane & anthracycline	30
		Hormonal	29
		Trastuzumab	19
		No chemotherapy	1

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21 Downregulation of the type I insulin-like growth factor receptor by a chimeric single chain antibody *in vitro* and *in vivo*.

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Insulin-like growth factors (IGFs) stimulate breast cancer proliferation, survival, and metastasis via the type I IGF receptor (IGF1R). Thus, IGF1R could be an important target in breast cancer therapy. In this study we have used a humanized single chain antibody against IGF1R (scFv-Fc) to inhibit IGF action in breast cancer. We have previously shown that scFv acted as a full agonist in MCF-7 cells and activated IGF1R tyrosine kinase activity when MCF-7 cells were treated with scFv for 10 minutes. Furthermore, scFv did not block IGF-I's ability to activate IGF1R or to stimulate growth *in vitro*. In contrast, scFv has been shown to partially inhibit xenograft growth of MCF-7 cells in athymic mice.

The objective of this study was to examine the mechanism by which scFv inhibited tumor growth. In contrast to other humanized antibodies such as trastuzumab, scFv was unable to enhance antibody-dependent cell-mediated cytotoxicity (ADCC) in an *in vitro* cytotoxicity assay. Incubation of cells with scFv, but not IGF-I, downregulated IGF1R levels after 2 h and the levels were greatly reduced after 24 h. 24 h pretreatment of cells with scFv inhibited the ability of 5 nM IGF-I to activate IGF1R, rendered cells refractory to further proliferation by IGF-I, and inhibited anchorage independent growth. To confirm if our *in vitro* findings of downregulation of IGF1R levels by scFv was responsible for inhibition of tumor growth *in vivo*, we tested the effect of scFv on IGF1R levels in mice with MCF-7 xenograft tumors. Two mice with tumors received intraperitoneal (ip) injection of 500 µg of scFv and one mouse was injected with PBS as control. Tumors were biopsied and IGF1R was measured by immunoblot. scFv caused downregulation of IGF1R levels 18 h after treatment. The levels remained downregulated up to 72 h after injection of scFv compared to the levels in the mouse that received PBS. Densitometric analysis showed that at 72 h after treatment with scFv, IGF1R levels in xenograft tumor extracts were about 30% of that in tumor extract from the control mouse.

These results indicate that this chimeric antibody against IGF1R downregulates IGF1R levels *in vitro* and *in vivo* and may have future potential as anti-IGF therapeutic in breast cancer therapy.

22 Autologous dendritic cells loaded with HER2-derived antigen BA7072 (APC8024) stimulate specific immune responses in patients with metastatic breast cancer overexpressing HER2: a phase I/II study.

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Background: The HER2/neu protooncogene product represents a potentially useful target antigen for cancer vaccine approaches, such as those utilizing autologous antigen presenting cells (APC), including dendritic cells, to stimulate immune responses.

Methods: Autologous APC were collected by leukapheresis and loaded with BA7072, an antigen construct consisting of recombinant sequences from both the extracellular and intracellular domains of HER2 fused to human GM-CSF. For the resulting vaccine (APC8024), total mononuclear cell dose was escalated from 2X10e8 to 5X10e9 in 3 cohorts. Patients with stage IV breast cancer and IHC 3+ or FISH+ tumor testing for HER2 were eligible, and received APC8024 on weeks 0, 2, and 4. Immune responses to BA7072 and separate HER2-based sequences were evaluated on weeks 0, 4, 8, and 16 by multiple methods.

Results: 14 patients received vaccine and were evaluable for safety. Toxicities consisted primarily of fever and chills following infusion. No Grade 3/4 toxicities were observed. Immune responses induced by APC8024 and specific for the immunizing antigen (BA7072) as measured by lymphocyte proliferation or IFNg ELISPOT were detected in 7 of 8 patients evaluable for response. Quantitative analysis of tumor cells by immunomagnetic enrichment/flow cytometric assay was performed in pheresis product pre- and post-APC processing to evaluate the possibility of ex vivo tumor cell expansion in parallel with APC maturation. No samples showed an increase in number of tumor cells as a result of vaccine generation; mean count was 1.13 ± 0.51 tumor cells/10e6 mononuclear cells in the pre-APC pheresis vs. 0.18 ± 0.09 in the vaccine product. Finally, 1 PR was observed in 8 patients evaluable for response (13%).

Discussion: We conclude that autologous antigen presenting cells pulsed with BA7072 were extremely well-tolerated, can stimulate immune responses specific for HER2, and appear to have antitumor activity in metastatic HER2-positive breast cancer.

23 A blood test for breast cancer detection.

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Background: Mammography is the test of choice for breast cancer, yet 20% go undetected. Many serum markers are available for breast cancer but none diagnostic. Protein chip mass spectrometry is an innovative technology that searches for multiple differentially expressed proteins creating a profile of biomarkers. Our objective is to identify unique serum profiles in combination with a classification algorithm to enhance the detection of breast cancer.

Materials and Methods: At our institution, female patients are prospectively enrolled into the biomarker study and donate serum prior to treatment following an IRB protocol. Proteins were denatured in the presence of urea buffer and applied onto a chip surface with metal binding affinity. Surface Enhanced Desorption/Ionization (SELDI) Protein Chip® mass spectrometry was performed creating protein profiles for comparison.

Results: From October 2000 to April 2002, 92 female patients (50 cancer, 42 benign) enrolled into the study. SELDI data were analyzed and a decision tree algorithm was generated using the Biomarker Pattern Software for the classification of benign and cancer groups. 7 protein peaks classified samples and were labeled using the Biomarker Wizard software. Cross validation studies showed a cancer diagnosis specificity/sensitivity of 85/78%.

Conclusion: The combination of SELDI Protein Chip® mass spectrometry along with the classification algorithm reproducibly identifies protein profiles for detection of breast cancer. The sensitivity and specificity of this technique approaches mammography and if confirmed in a larger study offers women a simple blood test for cancer detection.

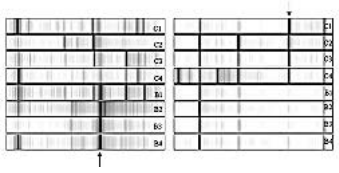

Figure 1. Gray scale ("Gel") views of two of the peaks employed in the generation of the decision tree. Peaks (blue arrow) and (red arrow) appear down-regulated and up-regulated respectively in the cancer (C1-4) compared to the benign (B1-4) samples.

24 High throughput global analysis of abnormalities associated with breast disease.

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Background: Breast cancer is the most diagnosed cancer in women and accounts for approximately 40,000 deaths in the United States of America annually. The molecular mechanisms associated with the onset, progression and severity of breast cancer remain largely unknown. As part of our molecular classification and biomarker discovery program, we routinely carryout high throughput "parallel" screening of breast tissue to identify abnormalities in DNA copy number and alterations in RNA and protein expression. The aim is to identify changes at the DNA, RNA and/or protein levels characteristic of disease category.

Methods: Breast tissues for this study were obtained from fully informed and consenting donors. RNA, DNA and proteins were isolated from whole tissue or by laser microdissection (LMD). Test tissue (IDCA, ILCA, DCIS or benign) and reference tissue (histologically normal) were labeled with Cy3 or Cy5 fluorescent dyes and signals captured with appropriate imagers. RNA profile was analyzed by microarray analysis of 3000 - 12,000 cDNA or oligonucleotide probes. Normalized signals are analyzed with GenoMax and Spotfire Decision Suite software's. DNA copy number was analyzed by comparative genomic hybridization (CGH) using DNA chips containing probes that target 287 regions that span the entire human chromosomes. Proteins were analyzed by 2-dimensional "differential in-gel" electrophoresis (2D-DIGE), using IPGphor IEF strips and 12% 24cm x 24cm polyacrylamide gels and signals analyzed with Decyder software. Differently expressed proteins are identified by mass spectrometry.

Results and Discussion: Microarray detected 2.5%-15% difference in transcript levels between normal and diseased tissue. DNA copy number differences were detected in each disease category. 2D-DIGE detected differential protein expression ranging from 8.0% (normal vs. normal) to 25.3% (benign vs. IDCA). 6% difference in protein expression was detected between cells obtained by LMD from normal and benign tissue. Parallel analysis of DNA, RNA and proteins isolated from the same tissue revealed far more molecular information. This presentation describes DNA copy number changes and alterations in RNA and protein levels associated with breast disease and discuss to what extent these technologies enhance molecular fingerprinting.

25 Ductal lavage findings in women with known breast cancer undergoing mastectomy.

Khan SA, Rodriguez N, Baird C, Ramakrishnan R, Wiley E, Nayar R, Bethke K, Staradub VL, Wolfman J, Morrow M. Feinberg School of Medicine at Northwestern University, Chicago, IL

BACKGROUND: The diagnosis of cancer in some patients with atypia on DL, and clinically normal breasts, has raised interest in its use as a cancer detection tool. This study examines duct lavage (DL) findings in women with known breast cancer, and the correlation of fluid yielding ducts (FYD) to the site of malignancy in mastectomy specimens.

METHODS: Women scheduled for mastectomy underwent DL in the operating room prior to surgery. If the DL sample was sufficient for cytological diagnosis, the lavaged duct was instilled with colored gelatin ex vivo. The breast was sectioned using a sub-gross technique, and histologic findings in areas with and without colored gelatin (dye) were recorded.

RESULTS: 27 women and 28 breasts have been entered; 23 had at least 1 FYD. At least 1 duct was cannulated and lavaged in 21 breasts, (mean # of ducts per breast 1.23). The lavaged duct matched the quadrant of the cancer in 14 breasts, and was located in a different quadrant in 7 breasts. Of the 12 cancer-containing mastectomy specimens analyzed so far, dye was present in the cancer-containing area of the breast in 6, and was present in areas of normal/benign breast tissue in 6 breasts. The main pathologic finding in 5 breasts with a dye/cancer mismatch was invasive ductal cancer. The major disease in 6 breasts where the dye matched the area of cancer was DCIS in 3, and invasive cancer in 3, but all of the latter were associated with significant amounts of DCIS. Lavage cytology data on the anticipated study set of 25 evaluable mastectomy specimens will be presented.

CONCLUSIONS: In the presence of a known cancer, the FYD is topographically related to the cancer in 67% of breasts, and can be traced to the cancer bearing area; this is accomplished more frequently when invasive cancer is associated with DCIS.

26 Hypermethylation of *BRCA1* promoter in sporadic breast cancer: comparison with *BRCA1*-associated hereditary breast cancer.

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Mutations of the *BRCA1* and *BRCA2* genes have been observed primarily in familial breast cancers or early onset disease. Several lines of evidence implicate the *BRCA1* and *BRCA2* genes in nonhereditary, sporadic tumors. Hypermethylation of the *BRCA1* promoter may be an important mechanism for functionally inactivating the gene in sporadic forms of breast cancers. *BRCA1*-methylated tumors display gene-expression profiles similar to *BRCA1*-associated hereditary breast cancers but the clinicopathologic features as well as the secondary genetic changes characterizing *BRCA1*-methylated tumors are not well delineated. **Methods**: *BRCA1* and Estrogen Receptor (ER) promoters were assessed in 5 breast cancer cell lines and 122 primary breast tissues by M-PCR. *BRCA1* expression was determined in 5 cell lines and 18 primary tissues by RT-PCR. In addition, we performed fluorescence in situ hybridization (FISH) using *BRCA1*, *HER2/neu* , and *MYC* probes and compared the results to *BRCA1*-associated hereditary breast cancer. **Results**: We observed *BRCA1* methylation in the UACC-3199 cell line and in 35 of 122 (29%) tumors. *BRCA1* methylation was correlated with reduction of chromosome 17 to one copy (monosomy) and down regulation or complete absence of the transcript. *BRCA1* methylation correlated with age of onset; 37% of tumors from cases under 55 years were methylated vs 20% of cases over 55 years ($p<0.005$). The methylated cases were equally distributed among all histologic types and there was no difference in the proportion of African American women (32%) vs non-Hispanic White women with methylated tumors (29%). The majority of *BRCA1*-methylated cases (79%) were ER(-) and/or ER methylated. *MYC* and *HER2/neu* amplification in methylated tumors were intermediate in values between hereditary *BRCA1*-associated and sporadic unmethylated tumors, suggesting that *BRCA1* methylation might be incomplete in some tumors. **Conclusion**: These results suggest that silencing of the *BRCA1* gene by methylation occurs in a significant proportion of sporadic breast cancers and may be an early event during tumor progression.

27 Breast surgery in the ATAC trial: women from the United States are more likely to have mastectomy.

Locker G, Sainsbury R, Cuzick J. The ATAC Trialists' Group

The ATAC trial randomized 9366 postmenopausal women from 21 countries, with early stage breast cancer, to adjuvant therapy with anastrazole, tamoxifen or the combination after the completion of primary surgery +/- radiotherapy +/- chemotherapy. Although there was an equal distribution of prognostic and therapeutic factors across the three treatment arms, there were differences in the mastectomy rates between countries represented. A retrospective analysis was done looking for features which predicted for having mastectomy (versus conservative surgery) to see if they could explain these national differences. In a univariate analysis ($p<.05$) tumor size ≥ 2 cm, node positivity (particularly ≥ 4), age over 69, poorly differentiated tumors and the use of adjuvant chemotherapy predicted for mastectomy. Having a tumor known to be estrogen receptor positive or patient weight greater than 70 kg predicted for breast conserving surgery. Patients entered onto ATAC from a study site, which enrolled ≤ 40 patients, were more likely to get mastectomy than those enrolled from sites, which entered more than 40 patients. Using the United Kingdom (n=3228, 42% mastectomy rate) as a standard; women from the United States were more likely to have a mastectomy (n=2222, 51%) with a hazard ratio of 1.43 (95% CI 1.28-1.60). In a multivariate analysis, being from the US remained an independent predictor for having a mastectomy. Although the standard of care in the United States remains breast conserving surgery whenever possible, in the ATAC trial, American women were more likely than those in the United Kingdom to have mastectomy. The reasons for this disparity are not clear and may represent physician or patient bias. If so, greater educational efforts should be made to support the role of conservative surgery as an alternative to mastectomy.

28 Cancer events after 18 years of follow-up in the treatment of early-stage breast cancer with mastectomy versus breast conservation therapy.

Poggi MM, Danforth DN, Sciuto LC, Smith SL, Steinberg SM, Liewehr DJ, Menard C, Lippman ME, Lichter AS, Altemus RM. NCI, Bethesda, MD; Univ. of Michigan Medical School, Ann Arbor, MI

Background: Although modified radical mastectomy (MT) and breast conservation therapy (BCT) offer similar overall and disease-free survival for patients with early-stage breast cancer, there are little long-term data on subsequent cancer events. With a median follow-up of 18.4 years, we present results from the randomized prospective NCI trial.

Materials and Methods: 247 patients with clinical stage I and II breast cancer were randomly assigned to modified radical mastectomy (116) or lumpectomy, axillary dissection and radiation therapy (121). Negative margins were not required. 237 patients have been followed for a median follow-up of 18.4 years. Cancer events were recorded as local, regional and/or distant. An isolated contralateral breast cancer in the MT or BCT group was considered a second primary as were other non-breast cancer histologies.

Results: Of 121 BCT patients, 27 (22%) experienced isolated ipsilateral in-breast events. After excluding salvaged in-breast events (16 patients), there is no significant difference in survival after a second event between MT and BCT ($p=0.22$). Among all patients, after censoring successfully salvaged events, there is no difference in the probability of second events between the two arms ($p=0.63$). At 15 years, there is no difference in the incidence of isolated contralateral breast cancer between BCT and MT ($p=0.92$). We found no difference in non-breast secondary cancers between the two groups ($p=0.67$). While long-term overall survival is not significantly different between groups after the development of metastatic disease ($p=0.072$), BCT patients had a lower probability of survival in the first year after distant failure (59% [CI 42-75] v. 97% [CI 84-99]).

Discussion: MT and BCT offer similar overall and disease-free survival despite a notable long-term incidence of isolated ipsilateral in-breast events with BCT.

29 Omission of radiotherapy after breast conserving surgery adversely impacts survival in elderly women.

Truong PT, Bernstein V, Speers C, Olivotto IA. Breast Cancer Outcomes Unit, British Columbia Cancer Agency, Vancouver Island Centre; University of British Columbia, BC, Canada

Purpose: Most trials showing benefits of radiotherapy (RT) after breast conserving surgery (BCS) have included few elderly women aged >75. This study analyzes survival according to age and RT use.

Methods: Data from the Breast Cancer Outcomes Unit, British Columbia Cancer Agency, were analyzed for 5557 women aged 50-89 referred from 1989-1998 with T1-2, M0 invasive breast cancer treated with BCS. Tumor, treatment characteristics and 5-year overall, breast cancer-specific, and local relapse- free survival (OS, BCSS and LRFS) were compared between women treated with RT vs. without RT (RT vs. NRT) for 3 age cohorts: 50-64 (n=2694), 65-74 (n=1912) and >75 (n=951). Median follow up was 6.4 years.

Results: RT was more frequently omitted with advancing age (12.7%, 13.6 % and 31.8 % in ages 50-64, 65-74 and >75 respectively, p < .0001). The NRT group had similar proportions of T2 tumors (24.1% vs. 22.3%, p=.62), lymphovascular invasion positive disease (25.0% vs. 26.1%, p=.54) and estrogen receptor negative disease (19.4% vs. 18.2%, p=.72) and fewer grade III disease (25.7% vs. 28.4%, p=.02) compared to the RT group. Among women aged >75, those not treated with RT had higher rates of surgical margin involvement (19.2% vs 10.3%, p=.0007), more local relapse (6.0% vs. 1.4%, p<.0001) and lower 5-year BCSS (85% vs. 93%, p<.0001) despite more frequent use of adjuvant tamoxifen (62.5% vs 43.8%, p<.0001). Lower 5-year OS and LRFS were also found among NRT compared to RT women in each age cohort (Table 1).

Conclusion: The use of post-BCS RT significantly declined with advancing age. Despite similar tumor characteristics and higher rates of tamoxifen use, women aged >75 not treated with RT experienced lower BCSS compared to their counterparts who received RT. These findings support the hypothesis that definitive local therapy impacts survival among elderly women.

5-year Kaplan Meier survival according to age and RT use

	No RT	RT	p
5-year Overall Survival			
age 50-64	.86	.92	.0003
age 65-74	.80	.89	<.0001
age >75	.58	.82	<.0001
5-year Breast Cancer-Specific Survival			
age 50-64	.93	.94	.14
age 65-74	.92	.93	.39
age >75	.85	.93	<.0001
5-year Local Relapse-Free Survival			
age 50-64	.94	.97	.004
age 65-74	.94	.98	.01
age >75	.93	.98	<.0001

30 Estrogen receptor expression as a predictive marker of the effectiveness of tamoxifen in the treatment of DCIS: Findings from NSABP Protocol B-24.

Allred DC, Bryant J, Land S, Paik S, Fisher E, Julian T, Margolese R, Smith R, Mamounas T, Osborne CK, Fisher B, Wolmark N. Baylor College of Medicine, Houston, TX; University of Pittsburgh, Pittsburgh, PA; and the National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, PA.

Background: From 5/91 to 4/94, 1804 women diagnosed with DCIS were recruited to NSABP Protocol B-24, and were treated with lumpectomy and radiation therapy (50 Gy) and randomly assigned to receive placebo (n=902) or tamoxifen (20 mg daily for 5 years, n=902). Results first published in 1999 demonstrated a conclusive benefit for patients receiving tamoxifen (incidence rate for all breast cancer = 18.3 per 1000 patients per year compared to 29.3 per 1000 patients per year, relative risk [RR]=0.63, 95% CI= 0.47-0.83, p=0.0009). However, estrogen receptor (ER) status was not a prerequisite for B-24, so that it was not known whether the benefit was restricted to ER-positive tumors.

Methods: ER status was determined for 628 patients (327 placebo, 301 tamoxifen) either by central immunohistochemical (IHC) assay of available material or by review of documentation from accruing sites. Cox proportional hazards models tested the effectiveness of tamoxifen vs. placebo in the cohort of women whose tumors were ER-positive and the cohort of women with ER-negative tumors, as well as the ER-by-treatment interaction.

Results: 482 of the tumors (77%) were ER-positive. In these tumors, the effectiveness of tamoxifen was clear (all breast cancer: RR=0.41, 95% CI=0.25-0.65, p=0.0002). Significant reduc-tions in incidence were seen in both the ipsilateral and the contralateral breast. In women with ER-negative tumors, little benefit was seen (RR=0.80, p=0.51), but the total number of events in this cohort was too small (n=36) to rule out a small, clinically meaningful benefit (95% CI for RR = 0.41-1.56). The test for ER-by-treatment interaction was suggestive but not significant (ratio of RRs=0.51, p=0.11). Institutional assays were more frequently ER-negative than central IHC assays (p=.016), raising the concern of false-negative results from laboratories using diverse non-standardized tests.

Discussion: These data suggest that ER expression is an important predictor of response to tamoxifen in patients with ER-positive DCIS. The results for ER-negative DCIS are inconclusive, pending additional data from this study (in progress) and other similar studies.

31 Local recurrences after DCIS therapy: diagnosis, treatment and outcome.

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Introduction : Local Recurrence (LR) after therapy for patients with DCIS can be a serious event, sometimes converting a previous stage 0 disease into life- threatening disease. This study assesses the outcome of salvage treatment according to initial therapy modalities.

Material : From 1985 to 1996, we analysed 1672 women with pure DCIS treated in 11 French Cancer Centers by mastectomy (M), conservative surgery alone (CS) or CS and radiotherapy (CS+XRT). Median age was 53 years and median follow-up after first surgery and LR diagnosis were 83 and 44 months respectively.

Results: 213 LR occurred (90 in situ and 123 invasive), with 55, 37 and 53-month median delays in M, CS and CS+XRT groups. 83% of in situ LR were discovered only by mammography, whereas more than 50% of invasive LR were discovered by clinical symptoms. Only one out of 90 women developed metastases after in situ LR (1.1%), whereas metastasis rates after invasive LR were 40%(2/5), 9.4% (5/53) and 16.9% (11/65) in M, CS and CS+XRT groups.

Local recurrences according to treatment modalities

	M (306)	CS (403)	CS+XRT (812)
ALL LR	5 (1.6%)	105 (26%)	103 (12.7%)
IN SITU LR	0	52	38
INVASIVE LR	5	53	65
AXILL. REC.	1	9	14
METASTASES	2 (0.6%)	5 (1.2%)	11 (1.3%)

Conclusion : DCIS remains an extremely favorable disease and the global metastasis rate varies from 0.6 to 1.3% at 7 years. However, this risk increases up to 16% after invasive LR. XRT clearly decreases LR rates in all subgroups of patients, but a close follow-up seems mandatory in high risk groups (e.g. women under 40), with a bi-annual clinical and mammographic control.

32 Benign breast disease and the risk of subsequent invasive breast cancer: findings from the National Surgical Adjuvant Breast and Bowel Project's breast cancer prevention trial.

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Background: The role of benign breast disease (BBD) as a marker for the development of invasive breast cancer (IBC) and the quantification of the IBC risk associated with BBD is an important issue for study. The Breast Cancer Prevention Trial (BCPT) of the National Surgical Adjuvant Breast and Bowel Project (NSABP) represents one of the largest studies in recent history in which healthy women were prospectively followed and the diagnosis of all breast biopsies were systematically reported. Information from this study provides a unique source of data to study the relationship between the BBD and IBC.

Methods: The records of 11,786 women without a history of atypical hyperplasia or *in situ* cancer were identified from the BCPT cohort. BBD information came from the pathological reports collected for each biopsy performed. The incidence of IBC among women with BBD was estimated and the effect of Tamoxifen on IBC risk among women who develop BBD was evaluated.

Results: In the placebo group, the incidence of IBC among women with BBD was 17.97/1000 and was substantially elevated when compared to women without BBD. The relative risk (RR) of IBC comparing women with and without BBD was 4.08. Older women (> 49) with BBD were more likely to develop IBC than were younger women (<50). The incidence of IBC was 30.11/1000 in older women and 12.32/1000 in younger women. The relative risk of IBC comparing those with and without a diagnosis of BBD was statistically elevated in both older and younger women (RR=5.92 and 2.66 respectively). When compared to the rates in the placebo population, rates of IBC among women in the tamoxifen group were lower than those in the placebo population. Lastly, BBD was shown as a statistically independent marker for prediction of IBC after the adjustment of the age, treatment and predicted 5 years risk from Gail model (P<0.0001).

Discussion: This study indicates that the risk of developing invasive breast cancer is elevated among women with BBD even after controlling the common risk factors included in the Gail model. The elevation in risk is greatest among older women. Risk reduction by treatment with tamoxifen among those who developed BBD is evident for women in both younger and older age groups, but may be greatest for younger women.

33 Reduced incidence of metastases in breast cancer patients treated with preoperative hormone replacement therapy (HRT): a retrospective study in 1160 women.

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Background: Hormone replacement therapy (HRT) is very common in the western world for the treatment and prophylaxis of postmenopausal discomforts like hot flushes, osteoporosis, etc. Although there may be a slightly increased risk of breast cancer for longterm HRT-users, these patients were reported to have a lower mortality and a longer overall-survival in comparison to never-users.

Material and Methods: We examined 1160 patients between the age of 45 and 70 at diagnosis of breast cancer with and without HRT with regard to the incidence of distant metastases. 313 patients were premenopausal (mean 47.0±3.0y), 847 were postmenopausal (55.5±4.4y), 343 of them received HRT (group HRT+) and 504 patients did not (group HRT-). Patients of group HRT+ received estrogenes over a minimum of 12 months (mean 60 months).

Results: There were significant differences between the postmenopausal groups regarding tumor size (25±18 HRT- vs. 21±14 mm HRT+) and grading. Nodal status, S-phase fraction, and hormone-receptor status showed no differences. Surgical and systemic treatment were similar in all groups. Regarding the incidence of metastases patients of HRT- has significantly ($p<0.001$) more metastases to bone (14% patients HRT- versus 3% HRT+), lung (9 vs. 3%) and liver (9 vs. 4%) in comparison to patients of HRT+.

Discussion: It was shown in animal studies and in clinical trials that downregulation of bone metabolism (e.g. with bisphosphonates) may reduce subsequent bone metastases efficiently. Although other mechanisms may play a role, we assume that the incidence of bone metastases in breast cancer patients can be reduced by normalizing bone metabolism (soil) due to HRT and worsening conditions of tumor cell seeding.

34 Hormone replacement therapy containing progestins and given continuously especially increases breast cancer risk in southern Sweden.

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We have previously reported an increased risk for breast cancer with longer duration of HRT use.The tumour incidence related to different types of hormonal replacement therapy (HRT) was followed in the same population based cohort of 29508 women aged 25-65 when interviewed between 1990-92. At the end of the follow up in December 2001, the cohort constituted 298649 personyears. Slightly more breast cancer cases were seen 556 than expected 508.37 (SMR=1.09, 95% CI 1.00-1.19). About 3663 women had ever used HRT.

In a Cox regression models, time to breast cancer in relation to duration and type of HRT use was analysed adjusting for age at menarche, age at first full term pregnancy, parity, age at menopause, family history of breast cancer and age at interview. In women with a natural menopause a significantly higher risk was seen for longer duration (4 years and more) of combined contineous HRT use compared with never users, HR =3.68, 95% CI 2.14-6.34. Also breast cancer risk after shorter combined sequential therapy was significantly elevated, HR=2.81, 95% CI 1.57-5.08.Increased non significantly elevated risks were also seen for longer combined sequential HR=1.40, 95% CI 0.60-3.20, gestagen only, HR= 1.94, 95% CI 0.49-7.58 and estriol use HR=1.38, 95% CI 0.63-3.01.No increased risk was seen in women after 5 years of nonuse.When studying women who ever used only one type of HRT, even more elevated hazard ratios for gestagen containing preparations were seen with the highest risks seen for combined contineous and gestagen only therapy in women with at least 48 months of use.Use of estradiol without progestins did not increase breast cancer risk significantly. The cumulative risk for breast cancer after at least 48months of use of a progestin containing preparation for a 50 year old woman with a follow up of ten years was 7% compared with never users 2.0%. For estrogen only users the corresponding figure was 3%. In conclusion longer use of HRT containing progestins significantly elevates breast cancer risk while estradiol use not significantly elevates breast cancer risk. Continued use of progestins rendered the highest risks. The yearly risk of breast cancer for long term users of progestins is of the magnitude of half the risk of a BRCA1 mutation carrier.

35 Phase III comparative study of trastuzumab and paclitaxel with and without carboplatin in patients with HER-2/*neu* positive advanced breast cancer.

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Background: In the treatment of patients with HER-2/*neu* positive advanced breast cancer, the combination of trastuzumab and paclitaxel has been shown to be superior to paclitaxel alone. Given the evidence for synergy between trastuzumab and platinum analogues, we conducted a randomized Phase III trial, comparing the combination of trastuzumab, paclitaxel, and carboplatin (TPC) with trastuzumab and paclitaxel (TP) in HER-2/*neu* positive patients with advanced breast cancer, to evaluate efficacy and toxicity.

Patients and Methods: 194 patients were registered. The majority of patients were Caucasian (83%), ECOG performance status 0/1/2 were 60%/36%/4%, and the median age was 55 years (range, 32-82). No prior chemotherapy for metastatic disease was permitted but 40% of patients had adjuvant chemotherapy. Cardiovascular status was monitored. Trastuzumab dosing was a standard loading dose of 4 mg/kg followed by weekly 2 mg/kg, paclitaxel was administered at 175 mg/m^2 over 3 hours every 3 weeks, and carboplatin was administered at an AUC of 6 every 3 weeks.

Results: For this analysis 160 patients were evaluable for response and time to progression (TTP). Response rate (RR) was 57% with TPC vs. 38% with TP ($p<0.01$). In HER-2/*neu* 3+ patients RR with TPC was 67% vs. 37% with TP ($p=<0.01$) and TTP was 13 months with TPC vs. 7 months with TP ($p=0.002$). In HER-2/*neu* 3+ patients TTP was 17 months with TPC vs. 9 months with TP ($p=0.004$). There was an increased frequency in grade 3 and grade 4 hematologic toxicities with TPC vs. TP in terms of neutropenia (54% vs. 23%) and thrombocytopenia (8% vs. 1%). TPC also resulted in increased nausea (5% vs. 1%). There were no differences in terms of neurologic toxicities, neutropenic fever, asthenia, cardiovascular, or pulmonary toxicities. There were no toxicity - related deaths.

Conclusion: Trastuzumab+paclitaxel+carboplatin is superior to trastuzumab+paclitaxel in terms of both response and time to progression with acceptable toxicity.

Supported by Bristol-Myers Squibb, Plainsboro, NJ and Genentech, Inc., San Francisco, CA.

36 Phase III trial of capecitabine (Xeloda®) plus bevacizumab (Avastin™) versus capecitabine alone in women with metastatic breast cancer (MBC) previously treated with an anthracycline and a taxane.

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Bevacizumab (Avastin; BV) is a recombinant humanized monoclonal antibody to vascular endothelial growth factor that is being evaluated in combination with chemotherapy in several cancers including MBC. Objective response or disease stabilization was observed in patients in a Phase II trial of BV alone in previously treated MBC and therefore a Phase III trial was initiated in combination with chemotherapy. Methods: Women with progressive MBC who had received no more than 2 prior chemotherapy regimens for MBC and had previously been treated with both an anthracycline (A) and a taxane (T) were eligible. Patients who had received both A and T in the adjuvant setting were eligible if relapse occurred within 1 year of completing chemotherapy. Patients were randomized to either capecitabine alone (2500 mg/m2/day po for 2 weeks of every 3-week cycle) or capecitabine combined with BV (15 mg/kg IV q3w). Treatment continued until progression and patients randomized to the combination arm were eligible to continue BV therapy after progression either alone or in combination with other therapies. Crossover from the control arm was not permitted. The primary endpoint of the trial was time to progression as determined by an independent review facility. Secondary endpoints were safety, objective response rate/duration and overall survival. Results: 462 patients were randomized and preliminary pooled demographic and baseline data are available for the majority of patients. Median age was 51 years and 50% of patients were ECOG 0. At primary diagnosis, 50% were ER positive, 42% PR positive and 27% HER2 positive. Median time since diagnosis of metastatic disease was 1.2 years. 76% of patients had visceral disease. The analysis of study results should be available in September 2002 and will be submitted at that time.

101 Initial experience with a radiofrequency (RF), circumferential, vacuum assisted biopsy device for ultrasound-guided breast biopsies.

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Background: We evaluated the efficacy, ease of procedure and safety of a new co-axial biopsy needle (SenoCor 360, SenoRx, Aliso Viejo, CA) using RF energy to target and biopsy breast lesions with ultrasound guidance.

Materials and Methods: Following IRB approval, 20 patients scheduled for ultrasound-guided biopsy gave informed consent and were prospectively enrolled between October 2001 and March 2002. The biopsy device is comprised of a co-axial guiding cannula and a biopsy probe that incorporates an RF cutting tip, circumferential cutting ring and a 360 degree vacuum. The RF is used to penetrate breast tissue and cut lesion specimens. Parameters evaluated included breast composition, procedure time and number and weight of samples. Ease of positioning, penetrating and sampling breast tissue were assessed on a 5 point scale from 1 (very easy) to 5 (very difficult). Pathologists evaluated the adequacy of the specimen for histological diagnosis. Patient adverse effects were assessed during the procedure and 7-10 days following the procedure.

Results: Of 20 attempted biopsies, 18 were successfully completed and 2 had malfunctions precluding use during biopsy. In 82% of cases, the breast composition was moderately dense. Procedure time (from skin insertion to removal of device after the final specimen) was 1:32 to 17:00 minutes (mean 7:31 minutes). Lesions were all masses ranging in size from 7-28mm (mean 15mm). An average of 4 (range 2-7) specimens were obtained, which included complete excision of the ultrasound imaging features of 6 fibroadenomas. The average specimen weight was 85mg with a range of 50-180mg. Concordant histological diagnosis was rendered in all cases. The device was judged very easy (1 on the 5 point scale) to hold and penetrate breast tissue in 90% of cases. Lesion penetration and sampling were rated very easy in 70-80% of cases. There were no significant complications.

Discussion: Initial results demonstrated that the SenoCor 360 breast biopsy device was ergonomic, easy to use and provided excellent operator control of positioning during targeting and sampling. The samples, acquired quickly, were significantly (approximately 6 times) larger than those obtained from standard spring-loaded core needle devices.

102 Therapeutic excision of benign lesions with the hand-held mammotome: initial experience.

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Purpose: To evaluate the therapeutic value of ultrasound-guided (US-guided) hand-held (HH) Mammotome biopsy in the excision of benign lesions.

Materials and Methods: Between April 2001 and February 2002, following surgeons referral and patient consent, 21 patients scheduled for surgical excision of benign lesions were offered US-guided excision using the HH Mammotome (11- or 8-gauge). Complete excision of the ultrasound image was to be attempted. For 35% of the patients, a biopsy of the tumor bed was undertaken. Pathologists evaluated the adequacy of the specimens for histological diagnosis. Complications were recorded during the procedure and within a month following the biopsy. Complete excision and scar were examined respectively by ultrasound and physical examination between 1 and 12 months post-procedure. Patients satisfaction with the procedure and the scar was assessed using a phone survey, with satisfaction rated on a 10-point scale (1=poor to 10=excellent).

Results: Of 21 patients recruited, 23 lesions were excised. Twenty lesions were fibroadenomas, ranging in size from 6 to 28 mm (mean: 16,2 mm). Number of specimens excised ranged from 5 to 52 (mean: 19). For 11 lesions larger than 15 mm, the 8-gauge Mammotome was used. Microscopic residual tumor in the tumor bed was found in 4 of 8 tumor beds examined, all early in the procedure learning curve. At the time of submission, follow-up had ranged from 1 to 12 months. No residual tumor had been seen under ultrasound for 12 lesions. Ultrasound differentiation between hematoma and residual tumor was difficult to assess in 4 lesions due to short follow-up. For the other 7 lesions, patients could only be reached by phone and not examined. Procedure satisfaction was rated 5.5 - 10.0 (mean: 9.3) and scar satisfaction was 6.5 - 10.0 (mean: 9.5). For the 8 patients that had had a surgical excision of a benign tumor prior to this procedure, procedure satisfaction ranged from 9 - 10 (mean: 9.5) and scar satisfaction ranged from 9 - 10 (mean: 9.8). There were no significant complications, but all patients had post-biopsy hematomas that resolved spontaneously.

Conclusions: Our initial results demonstrate that US-guided therapeutic excision of benign lesions with the HH Mammotome is a reliable procedure with a very high rate of patient satisfaction.

103 Percutanous core-needle biopsy of palpable breast tumors: Do we need ultrasound guidance?

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Background: Percutanous core-needle biopsy has become widely accepted for preoperative his-tological examination of breast lesions. The purpose of this study was to evaluate the valence of sonography for core-needle biopsy of palpable breast lesions.

Material and Methods: In the present single institution study we analysed 170 breast lesions retrospectively. Percutanous breast biopsies were performed using a biopsy device (Bard TM) with 14-gauge needles. Two groups were analysed separatly: 82 biopsies were performed without ultrasound documentation of the procedure (group I) and 88 biopsies were done under continuous ultrasound guidance (group II). The diagnoses achieved by core-needle biopsy were compared with the surgical diagnoses of the tumors subsequently excised.

Results: In patient group II two false negative findings occurred (sensitivity 98%) and in group I 17 lesions were categorized as core breast biopsy cancer misses (sensitivity 78%). Among the 17 false negative lesions 13 lesions were 3 cm in mean diameter or smaller.

Discussion: Our data indicates that ultrasound guidance for percutaneous core-needle biopsy of palpable breast lesions is an indispensable part for the accuracy of this method.

104 First clinical experience combining mammography with a new high sensitivity, high resolution gamma imaging system for guided breast core biosy.

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Purpose: Evaluating the feasibility and the role of the combination of anatomic and physiologic information during stereo tactic breast core needle biopsy (cnb). Assistance guiding biopsy into viable malignant tissue. Decreasing the false negative rate of stereo tactic breast cnb.

Methods and Materials: Eight patients with mammographic abnormalities, categorized as BI-RADS IV and V were examined. The patients were injected intra-venously with 20mCi 99mTc-sestamibi and planar scintimammography was performed. The patients were then transferred to the mammography unit; scout and stereo tactic films of the suspicious lesion were taken, with simultaneous acquisition of using V-Target scans, preceding the 14g stereo tactic cnb.

The V-Target imaging system is a hand-held gamma detection element attached to a very wide-angle collimator and navigation system. Gamma photons accumulations are tracked with six degrees of freedom and analyzed by a patented image reconstruction algorithm. This advanced physiological imaging system offers an increase of 30-60 times in sensitivity compared to average, with spatial system resolution better than 3mm.

The results were compared with mammography, and planar scintigraphy, while histologic results served as gold standard.

Results: There was a spatial correlation between mammography results and physiology parameters obtained using the V-Target probe. In addition, physiologic imaging detected abnormalities not shown on mammography or conventional NM procedures. Lesions detected both with the V-Target probe and mammography, were not always seen on standard nuclear medicine cameras.

Conclusions: Anatomic and physiologic imaging fusion in breast cnb is feasible. Future use of V-Target imaging system for breast physiologic-guided biopsy, without the need of anatomic guidance should be investigated. V-Target images showed high sensitivity for physiologic activities in breast tissues, providing information previously not available using standard gamma cameras. Physiological identification of breast abnormal tissue using Sestamibi was examined and should be further evaluated, as part of the on going clinical study, in which a total of 80 patients are expected to be enrolled in the next few months.

105 Fine needle aspirate biopsies of breast lesions are more commonly unsatisfactory and inaccurate when performed by non-surgeons.

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Background: Fine needle aspirate (FNA) biopsy is a common technique for diagnosis of breast lesions. It is unclear whether FNA accuracy varies with type of clinician performing biopsy, and how this compares with other needle biopsy techniques.

Methods: To further study, we retrospectively analyzed 532 consecutive patients referred to a single practice with 588 needle biopsies of breast lesions, done at Cook County Hospital (CCH) in Chicago, which serves the medically-indigent.

Results: A total of 201 FNAs were performed; 139 (69%) were done by non-surgeons (primary-care physicians), with 77 (55%) producing inadequate or unsatisfactory samples; 62 (31%) were done by surgeons, with 19 (31%) unsatisfactory samples. For the 105 (52%) FNAs with satisfactory samples, the overall sensitivity, specificity and accuracy was 100, 80.2 and 81.9 respectively; these results were not significantly different for non-surgeons (62 FNAs - 100, 73.5 and 79) and surgeons (43 FNAs - 72.7, 90 and 86) respectively. A further 155 cases had clinical 16 gauge core-needle-biopsy (cCNB) performed by surgeons, and 232 more had image-guided (ig) CNBs done in radiology. There was no significant difference between cCNB or igCNB for unsatisfactory samples (2 vs. 1.3% respectively), or sensitivity, specificity and accuracy (93.2, 98.4 and 95.4 vs. 92.9, 99.3 and 97.3 respectively). Both CNB methods were significantly more accurate (13.44% difference) for diagnosis than FNA (95% CI: 21.4-5.5%; Z test p=0.001). The probability of an accurate result, compensating for unsatisfactory sampling, was calculated for each method (accuracy x satisfactory fraction); this was 35% for FNA done by non-surgeons, 59% for FNA done by surgeons, 93.5% for cCNB done by surgeons, and 96% for igCNB done by radiologists.

Discussion: FNA biopsy of breast lesions performed by non-surgeons are most likely to produce an unsatisfactory sample and least likely to produce an accurate result. Improved sampling and accuracy with breast needle biopsy can be anticipated with pre-biopsy specialist consultation for selection of appropriate biopsy technique.

106 Displacement of tumour cells in the needle tract following (vacuum assisted) core needle biopsy (VA)CNB of breast lesions: incidence and morphology.

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Aim: To determine the incidence of tumour cell displacement in the needle tract following needle biopsies and to correlate this with tumour morphology.

Materials and Methods: From March 2001 till March 2002, 240 preoperative wire localisations and 1086 invasive procedures of breast lesions were performed: 120 11G VACNB, 280 18G CNB, 62 14G CNB, 624 Fine Needle Aspiration Cytologies (FNAC). During gun-biopsy an average of 2 passages was made. During macroscopic examination of surgical excision specimens the needle tract was excised, embedded in paraffin and examined microscopically.

Results: A diagnosis of invasive carcinoma was made in 215 (VAC)NB; DCIS was diagnosed in 80 (V)CNB. In 6 cases displacement of malignant cells in the needle tract was seen in the surgical excision specimen. In 5 cases solitary cells or cells groups could be visualised in granulation tissue or in cellular fibrous tissue, mimicking an infiltrative pattern. In 1 case displacement of tumour cells into vascular spaces was noticed. In 4 cases the pre-operative diagnostic procedure was a 18G CNB with 3 passages, in 1 case a 14G CNB with 2 passages and in 1 case a 11G VCB. The definitive diagnoses in all these cases included extensive moderately to poorly differentiated DCIS. The maximum diameter of DCIS was 4,5 cm. Displacement of tumour cells was never noted after wire localisation or FNAC.

Conclusion: During a 1 year period, displacement of malignant epithelial cells was seen in 6 cases after (VA)CNB. Compared with the literature, this is a rather infrequent event. This low frequency cannot be attributed to the use of small gauge needles, since in 4 out of 6 cases, a 18G needle was used. However, it may be partly explained by the low number of passages performed in each case. Although not corroborated by literature, our results suggest that also the type of lesion present may influence tumour cell displacement. Indeed, in all 6 cases in which malignant cells were found in the needle tract, the diagnosis included an extensive moderately to poorly differentiated DCIS.

107 Atypical ductal hyperplasia on needle core biopsy: a correlation with open surgical biopsy findings.

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BackgroundStereotactic guide needle biopsy (SNB)is being used with increasing frequency to obtain pathological information on areas of mammographic concern.The aim of needle biopsy is to decrease the open biopsy rate in benign abnormalities and increase the preoperative biopsy rate in lesions which require open surgical biopsy.thus minimising unecessary open biopsy and the number of surgical proceedures required in each individual Atypical Ductal Hyperplasia (ADH) is a pathological diagnosis associated with an increase risk of breast carcinoma,its significance on SNB is of some controversy.**Design**Between 1996 and March 2002 49 SNB's yielded the diagnosis of ADH.These biopsies were of areas of suspicious or indeterminate microcalcification,in women aged 50-65 years,using a 14 gauge automated core biopsy.Pathological features at open biopsy were reviewed.We aimed to assess if Atypical Ductal Hyperplasia on SNB warranted subsequent open surgical biopsy**Results**Of the 49 individuals with SNB 47 underwent open surgical biopsyAt open biopsy,.22 cases (45%) showed Ductal Carcinoma insitu,11cases(22%) invasive breast carcinoma,2 cases (4%)Lobular carcinoma insitu,6 cases (12%)Atypical ductal hyperplasia and 6cases(12%) benign simple hyperplasia.Thus of the 47 cases with ADH on SNB 67% were upgraded to more significant pathology at open biopsy.**Conclusion**These results suggest that in our institution the SNB diagnosis of Atypical Ductal Hyperplasia is associated with a high rate of conversion to a more significant lesion at open surgical biopsy,particularly of Ductal Carcinoma insitu .Therefore we must advise that all cases with the SNB diagnosis of Atypical Ductal Hyperplasia should be refered for open surgical biopsy to determine the presence of any more sinister findings.

108 Obtaining the preoperative diagnosis of breast cancer with minimal requirement for open surgical biopsy.

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Background: The preoperative diagnosis of breast cancer generally allows informed discussion followed by the performance of a single definitive surgical procedure. Our current approach to achieving the preoperative diagnosis of breast cancer is usually FNA for palpable lesions. Standard needle-core biopsy was predominantly used for impalpable lesions. The success of this approach was determined.

Materials and Methods: A prospectively accrued database was analysed for all patients diagnosed with invasive or non-invasive breast cancer between January 1997 and December 2000. Our aim was to identify the preoperative diagnosis rate, and the accuracy of core biopsy in differentiating pure DCIS and invasive cancer.

Results: Over the 4 year period, 583 of 616 cases (95%) of breast cancer were diagnosed preoperatively. Invasive cancer was more frequently diagnosed preoperatively (503 of 519 cases, 97%) compared to pure DCIS (80 of 97 cases, 82%). The method of diagnosis was FNA 261 cases, core biopsy 319, open surgical biopsy 32, nipple discharge cytology 1, clinical diagnosis 2, and one patient had a frozen section and proceeded to the definitive resectional procedure.

Core biopsy was incorrect in differentiating pure DCIS and invasive cancer in 14 patients with invasive disease on final histology. Conversely, 9 patients with pure DCIS had an axillary dissection based on the incorrect core biopsy or FNA diagnosis of invasive cancer

Discussion: A high level of preoperative diagnosis can be achieved with FNA and standard core biopsy. FNA and core biopsy are more sensitive in diagnosing invasive cancer compared to DCIS.

Our approach to the diagnosis of breast cancer meant that only 5% of patients required an open surgical biopsy to diagnose their palpable or impalpable breast cancer. This reduced need for surgical biopsy would also reduce the total number of surgical procedures required.

109 Ductal cells, hyperplasia and breast cancer risk: results from a long-term follow-up study of lavage patients.

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Background: Ductal lavage, or the collection of ductal fluid with a microcatheter, has received much attention recently as a potentially accurate technique for risk stratification of high-risk women. Recent studies have not however had a long enough follow up period to estimate breast-cancer risk according to the cellular composition of ductal fluid.

Methods: We conducted a follow up study of 414 patients identified to date that received ductal lavage in the 1970's and 1980's by the late Otto Sartorius, MD, a clinician specializing in breast disease, who practiced in Santa Barbara, California. Ductal lavage generally followed injection of contrast material as part of the ductogram procedure, and consisted of flushing the ducts and collecting the resulting fluid. Clinical records of lavage patients originated from the Santa Barbara Breast Cancer Institute and Goleta Valley Hospital. Data were collected by two separate teams of abstractors. We mailed invitation letters containing an enclosed informed consent, with study questionnaires sent to consented subjects. We categorized ductal epithelial cells as follows: insufficient cells to make a diagnosis, normal, fibrocystic, hyperplasia, atypical hyperplasia including papillomatosis. We estimated risk using age-adjusted poisson-regression models.

Results: Among the 191 women thought to be alive with deliverable addresses, to date, 42% consented to participate in follow-up, with 56 women returning a questionnaire. Mean and median follow-up time was 19.6 and 19.7 years respectively. Age-adjusted risk of developing breast cancer associated with hyperplasia was 1.29 (95% CI=0.50 - 2.72), and atypical hyperplasia was 2.32 (95% CI = 1.01-4.41). Fibrocystic, normal and insufficient cellular categories were inversely associated with risk.

Discussion: We demonstrated, using a well-characterized clinical lavage sample, increased risk of breast cancer with hyperplastic and atypical hyperplastic breast epithelium cells after almost 20 years of follow-up.

110 Ductal lavage using aseptic technique in a series of 100 high-risk women.

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Objectives: The study was conducted to evaluate the feasibility of performing ductal lavage aseptically (clean no touch) in a surgical practice. All previously published ductal lavage reports were performed under sterile technique.

Methods: One hundred fourteen high-risk patients (prior breast cancer, elevated Gail risk, or pathologic nipple discharge) with clinically negative mammograms and no palpable masses. Each NAF producing duct was cannulated using the FirstCyte™ Breast Microcatheter. The Microcatheter tip and dilator tips were kept sterile. Dilators were cleansed with alcohol and maintained on sterile gauze between duct cannulations. After the catheter was properly seated, ductal lavage was carried out as previously described. Each fluid-yielding duct was lavaged using a separate catheter. All specimens were put in CytoLyt® solution and cytologically analyzed.

Results: From March, 2001 to May, 2002, 114 patients underwent nipple aspiration. Of the patients, 32 did not undergo lavage: 26 (22.8%) did not produce NAF and in 3 (2.6%) patients the duct could not be cannulated. Among the 82 patients who underwent lavage, 45 (54.9%) patients had benign cytological results, 20 (24.4%) patients revealed abnormal cells, 18 with mild, 1 with marked atypia and 1 patient with malignant cytology. 17 (20.7%) patients had insufficient epithelial content for analysis. No woman in this series reported symptoms consistent with infection (warmth, redness, persistent breast pain) post lavage.

Conclusion: In a series of more than 100 women at high risk of developing breast cancer, ductal lavage detected abnormal cells in 24%. Ductal lavage was performed safely and without infection using aseptic technique.

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ABSTRACT #111

WITHDRAWN

112 Ductal lavage: initial 18 month experience at a single institution.

Willey SC, Rezaei K, Hegde P, Berezowski K, Brem R, Sidawy M. George Washington University, Washington, DC

Introduction: Breast ductal lavage (DL) is an additional risk-stratification tool for women at high risk for developing breast cancer. It is a method of obtaining ductal epithelial cells by cannulating the ductal system and performing lavage with saline solution.The cells are then examined cytologically.

Material and Methods: From November 2000 to April 2002, 39 women (age range 27-79 years) at high-risk for developing carcinoma of the breast (Gail index ≥ 1.7%, previous history of breast cancer, BRCa1 or 2 positive) or with nipple discharge underwent DL. The DL specimens were processed by the ThinPrep® method and classified as insufficient, benign, mild atypia, marked atypia and malignant.

Results: A total of 90 DL specimens were examined from 39 patients. The number of lavaged ducts ranged from 1-5 per patient. Cytology results were: insufficient 21(23.3%), benign 65 (72.2%) and marked atypia 4 (4.5%). No mild atypia or malignant diagnoses were rendered. Surgical follow-up was available in 3 out of 4 patients with marked atypia, and revealed an intraductal papilloma (1) and ductal carcinoma *in situ* (2). All 3 patients presented with bloody nipple discharge. The remaining patient with marked atypia was asymptomatic. She had a negative mammogram, sonogram, MRI and ductogram and repeat DL was benign. She has not had surgery and is being followed closely. One patient with benign cytology results from DL performed for clear nipple discharge underwent biopsy of the ipsilateral breast six months later for increasing calcifications on mammography and had ductal carcinoma *in situ*

Conclusions: These results demonstrate that DL recovers sufficient samples for cytologic interpretation in 77% of patients. Marked atypia was associated with intraductal pathology in 3 of 4 patients for whom surgical follow-up is available. Prospective clinical studies are in progress to evaluate this technique further.

113 Exfoliative breast cytopathology: An experience with ductal lavage.

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Background: Ductal lavage (DL) has been successfully used to sample cells from the entire ductal tree. Hence, this technique can be used to detect ductal and lobular breast carcinomas in high-risk patients. In conjunction with the newly identified genetic markers, this technique can prove to be a powerful diagnostic tool for early breast cancer diagnosis before any mammographic changes occur.

Design: Unilateral or bilateral Nipple Aspirate Fluid (NAF) and DL were performed on 20 high-risk women, aged 23 to 57 years old, in which no abnormality was detected by breast exam and mammography. Specimens were processed using the Thin Prep protocol. Upon subsequent cytological examination, one of the following four categories for diagnosis (benign, atypia, malignant or insufficient) was assigned to each specimen.

Results: Among the 20 high-risk patients, 6 patients underwent bilateral or unilateral multiduct lavage. As a result, a total of 24 DLs were performed on the 19 patients. 4 (16.7%) were deemed insufficient while 19 (76.1%) yielded a benign diagnosis. 1 DL (4.2%) was assigned atypia, and the patient was advised to undergo galactography for further evaluation (result pending), patient from the group of 20 was found to have atypical cells in NAF upon initial examination and a subsequent biopsy showed evidence of ductal carcinoma *in situ.* No DL was performed on this patient.

Conclusion: All women tolerated the procedure well and the entire study was completed without any complications. The number of insufficient specimens was far less in DL, compared to NAF. Thin prep processing protocol proved to give excellent results. After the necessary cyto-diagnostic standardization and correlation studies, we believe that this technique, when combined with specific cytogenetic markers, can be a powerful diagnostic tool for early breast cancer detection and in stratifying high-risk population.

114 Correlation between the biofield diagnostic system test and Ki-67 labelling index to identify highly proliferating palpable breast lesions.

Gatzemeier W, Scelsi M, Galetti K, Villani L, Tinterri C, Regolo L, Costa A. Italy; Maugeri Foundation, Pavia, Italy

Introduction: The Biofield Diagnostic System (BDS) is a non-invasive device that aims at providing an objective measurement of proliferation rates by comparing electropotentials in a quadrant of the breast containing a suspicious lesion with the net electrical activity and that of uninvolved segments with sensors placed on the skin surface. A retrospective analysis demonstrated a 86% concordance between BDS and Ki-67 in discriminating highly proliferate lesions. **Patients and Methods:** In order to further confirm these findings 100 patients with palpable breast lesions who were scheduled for open biopsy were enrolled into a prospective study to correlate preoperative BDS results with those of the definitive pathological diagnosis including the Ki-67 expression, grading, receptor status and other prognostic indicators. The data set for each patient consisted of individual Biofield electrical variables and tumour characteristics describing the lesion. Tumour grading was scored according to Scarff-Bloom-Richardson . Up to five Ki-67 evaluations (DAKO A/S, Goldstrup, Denmark) were performed on each patient. Average Ki-67 antigen expression values were compared to the Biofield index using logistic regression. All analysis were performed using SAS statistical software (Cary, NC). **Results:** Evaluation of the data showed a statistically significant correlation between the BDS indices and the high and low Ki-67 labelling indices as well as between increasing Ki-67 and BDS indices and with tumour grading characteristics. **Conclusion:** These results suggest that BDS is an accurate, non-invasive method to measure proliferative activity in suspicious breast lesions preoperatively. Such information can be useful for the decision making process and may influence the individual treatment strategy in women with primary breast cancer. The diagnostic results are objective and immediately available. The BDS is safe, simple to perform, acceptable to women and cost-effective.

115 Localization of breast lesions with radiolabeled vitamin B12 analog (In-111 DAC): preliminary safety and imaging results.

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Background: Cellular uptake of vitamin B12 or cobalamin (CBL) is increased in rapidly dividing malignant cells. This action may be mediated through up-regulation of the transcobalamin II receptors and the carrier protein responsible for cobalamin transport within the intravascular/extracellular space. Intravenous human studies with DAC (diethylene-triaminepentaacetate adenosylcobalamin), a CBL analog, labeled with the gamma emitter Indium-111, have demonstrated the feasibility of imaging a variety of malignancies. A Phase I trial to evaluate DAC as an imaging agent of breast tumors is underway.

Materials and Methods: This is a non-randomized trial to evaluate safety and imaging performance characteristics of In-111 DAC in 12 patients with known or suspected (clinically or by diagnostic medical imaging) cancers of the breast. To date, 8 patients have been enrolled. After signing an informed consent and undergoing a pre-study screening evaluation (including physical exam, resting ECG, liver, renal function tests, blood counts and urine analysis), each patient received approximately 650 mCi of In-111 DAC intravenously. Both breasts and axillary regions were evaluated by planar and SPECT imaging at 30-120 minutes, 3-7 hours, and 20-26 hours. Tissue diagnosis (biopsy, lumpectomy) was performed after DAC injection.

Results: Malignant lesions (3 patients) were characterized by focal and persistent increased In-111 DAC localization whereas benign fibrocystic lesions were characterized by diffuse, low grade activity which declined with time. No adverse reactions were observed and no toxicities reported.

Discussion: Our preliminary findings indicate the feasibility of targeting malignant breast lesions with In-111 DAC. In addition, this novel imaging agent appears to be safe and easy to use.

116 Utility of biopotentials measured with the Biofield Diagnostic System for distinguishing malignant from benign lesions and proliferative from nonproliferative benign lesions.

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Background: This report is a new study of DC voltage differentials conducted to examine deplorization of breast epithelium as described by Cuzick, et al. (Lancet 1998; 352:359). Biofield Corp. subsequently improved the device, sensors and feature selection for determining direct current voltage differentials. A multinational study was completed in 19 centers. After CE Marking, PostMarket Surveillance (PMS) was instituted to survey the performance in a routine clincial practice.

Material and Method: Biopotentials were measured on the surfaces of the breasts in 1839 patients with suspicious lesions using the Biofield Diagnostic System (BDS). Palpable and nonpalpable breast lesions were studied in patients aged 18.8 to 90.7 years. Proliferative activity was measured by Ki-67 antigen expression in 365 cancers. The clinical management system was designed to produce a sensitivity of at least 95 out of 100 with a specificity of greater than 60 out of 100. Estimates were made retrospectively to determine the number of saved biopsies had the BDS been applied.

Results: Invasive breast cancer was found in 592 patients, 89 patients had DCIS, and 602 patients had proven benign lesions. In 816 patients, biopsy was not considered indicated. There was a significant ($p<0.05$) correlation of the biopotential differentials according to the level of aggressiveness of malignant lesions. The biopotentials measurements in the breasts were significantly ($p<0.05$) higher in the proliferative benign lesions than in the nonproliferative benign lesions. It was estimated that more than 40 out of 100 breast biopsies could have been spared using the BDS. In the survey of more than 250 results from routine clinical practices (PMS), the sensitivity of the tests was >95 out of 100.

Discussion: The BDS provided an accurate, objective measurement of the aggressiveness of malignant breast lesions and of the level of proliferation of benign lesions. The test was noninvasive, cost effective and acceptable to women.

117 Correlation of clinical and pathological stage in breast cancer.

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Objective: To correlate the clinical stage with the pathological stage in breast cancer and to estimate the rate of discordance.

Method: A five-year retrospective analysis was done from the tumor registry data from the patients diagnosed with breast cancer, in relation to their clinical and pathological stages.

Result: A total of 883 patients' data diagnosed with breast cancer was analyzed. Discordance between clinical and pathological stages was noted in 221 patients (25%). Further analysis of the discordant data showed that 160/221 (72%) patients had undocumented and/or unavailable clinical stage. They were staged appropriately after definite treatment. Only 61/221 (28%) had true discordance between the documented clinical and pathological stages. Details of the true discordance revealed that 29/61 (48%) were upstaged and 32/61 (52%) were downstaged. Of the 32 patients that were downstaged, 15 patients (47%) received neoadjuvant chemotherapy. Tumor response was the cause of downstaging in these patients.

Conclusion: In the era of health care cost constraints, expensive work up for staging breast cancer can be minimized. With the discordance rate of only 8.4% (61/723) in our study, we can reemphasize the fact that meticulous clinical examination can still be used with confidence for treatment planning and prognosis.

118 Characterization of benign versus malignant breast lesions with arcitumomab: technical considerations.

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Background: Arcitumomab (CEA-Scan, Immunomedics, Morris Plains, NJ) a murine IgG1 monoclonal antibody specific for CEA, labeled with the gamma emitter Technetium-99m, has been shown to localize in breast carcinomas with a sensitivity of 61% and a specificity of 91% (Cancer 2000, 89:104-15). In a subset of patients, sensitivity was further improved through image-enhancement techniques which will be described in this study.

Materials and Methods: A total of 49 patients were entered in the study because of a mammographic/ultrasonographic abnormality (n=32) or histologically confirmed breast carcinomas (n=17). Patients were imaged (planar, tomoscintigrams, and lateral prone dependent views) 3-4 hours following IV Arcitumomab administration. Computer-enhanced darker intensity views of the axillae, lateral prone views of the breast and SPECT images were independently analyzed.

Results: Arcitumomab scans correctly identified breast cancers in 13/17 patients, missing 4 lesions less than 1.0 cm in diameter. Lesions were clearly delineated against a faint background. Enhanced axillary view increased detection for lymph node metastases from 55% to 73%. No false-positive results were encountered. Compared to anterior supine views, lateral prone dependent views of the breast led to false-positive findings in 7 of 18 patients with benign non-proliferative breast disease.

Discussion: Using computer assisted tumor to background enhancement techniques, we were able to increase the detection rate of lymph node metastases with Arcitumomab. Furthermore, because of higher false positive rates, we do not recommend the use of lateral prone dependent breast views. Arcitumomab is an easy to use, safe, non-immunogenic imaging agent with adequate imaging performance characteristics, particularly its high specificity and positive predictive values.

119 Adverse impact of lymphangitic and vascular invasion (LVI) in early breast cancer: results from the British Columbia (BC) breast cancer outcome unit.

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Between 1989-1998, 12,951 stage I-II breast cancer patients were treated at the BC Cancer Agency with adjuvant therapies according to the evolving BC Breast Tumor Group guidelines. Of those, 10,104 had information on LVI (7,031 node negative, N-ve; and 3073 node positive, N+ve). Outcome was 10 year survival free of breast cancer recurrence (10 y DFS); median follow up was 5.4 years. The LVI impact was examined in a multivariate analysis adjusted for tumor size, grade, ER status, age, therapy (adjuvant radiation, chemotherapy, hormones) and nodal status (for N+ve cases).

RESULTS:

	LVI -ve	LVI+ve	RR (95% CI)	p
Node-ve, unadjusted	83.4%	77.2%	1.75 (1.38,2.21)	<0.001
Node-ve, adjusted			1.75 (1.03, 1.45)	<0.001
Node+ve, unadjusted	69.4%	56.9%	1.63 (1.40, 1.89)	<0.001
Node+ve, adjusted			1.22 (1.03, 1.45)	0.020

CONCLUSION: Our data indicate that the presence of LVI confers a significantly higher chance for breast cancer recurrence, both in N-ve and in N+ve cases, independent of other prognostic and treatment factors. As a result, different therapeutic approaches should be examined for the LVI cohort, and trials of new adjuvant regimens should consider stratification for LVI (in addition to nodal status, ER status, age, etc.).

120 Lympho-vascular invasion (LVI) and prognosis in invasive breast cancer.

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The prognostic power of micrometastases in lymph nodes (LN) is currently under re-evaluation. Another marker of early lymphatic spread is lympho-vascular invasion (LVI). This was assessed on haematoxylin and eosin-stained sections at the periphery of 3931 primary operable invasive breast cancers in women presenting to the Nottingham Breast Unit between 1973 and 1998. Definite vascular invasion was categorised as positive and probable or absent vascular invasion as negative. Lymph node status was determined by an axillary sampling surgical procedure and each node carefully examined histologically. Long term follow-up and tumour and patient characteristics were recorded.

Of the total cases, 75% showed no LVI and 25% were classed as LVI+. Forty three % of patients with LN+ disease showed LVI compared with only 17% of 2309 LN- cancers ($p<0.001$).

In lymph node negative breast cancer the 10 year overall survival was 67% in LVI+ disease and 79% for LVI tumours ($p<0.001$). Multivariate analysis in LN cancers with histological grade, invasive tumour size and LVI entered, showed that LVI retained independent prognostic significance. Beta co-efficients for grade, size and LVI were 6.9, 10.0 and 3.1 respectively.

In conclusion, LVI appears promising in adding prognostic discrimination in lymph node negative invasive breast cancer.

121 The prognostic and predictive value of polymorphonuclear leukocyte elastase in primary and advanced breast cancer.

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Background: A variety of serine proteases, including urokinase-type plasminogen activator (uPA), plasmin, and polymorphonuclear leukocyte elastase (PMN-E), have been implicated in the processes of tumor cell invasion and metastasis. Besides degrading of matrix proteins, PMN-E has been shown to be able to cleave and inactivate plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of uPA, and α2-antiplasmin, the natural inhibitor of plasmin, thus enabling uncontrolled matrix degradation by the fibrinolytic enzymes.

Materials and Methods: Using ELISA, the levels of PMN-E, uPA and PAI-1, were determined in 1143 cytosolic tumor extracts of patients with primary breast cancer, and of 387 and 76 advanced breast cancer patients who received tamoxifen or chemotherapy, respectively, as first-line treatments. In patients with primary breast cancer, their levels have been correlated with the lengths of relapse-free survival (RFS), distant metastasis-free survival (DMFS), and overall survival (OS), and in patients with advanced disease, with the type of response to treatment, and the lengths of progression-free survival (PFS) and post-relapse overall survival (PROS).

Results: Patients with high PMN-E levels in their primary tumor had a poor RFS, DMFS, and OS, especially during short-term follow up (0-60 months). This held true for node-negative and node-positive subgroups of patients as well. In multivariate analysis, PMN-E added to the information provided by the traditional prognostic factors, in the analysis for DMFS even after correction for uPA and PAI-1 as well. In patients with advanced disease, high PMN-E tumor levels were associated with a poor type of response to tamoxifen therapy, also after correction for the traditional predictive factors. Furthermore, high PMN-E levels were associated with a poor PFS and PROS. No such relationships were observed for patients treated with chemotherapy.

Conclusion: Our present results suggest that PMN-E is associated with breast cancer metastasis and that the tumor PMN-E status might be helpful in selecting the appropriate individualized (adjuvant) treatment for patients with breast cancer.

122 Prognosis of infiltrating lobular versus infiltrating ductal carcinoma of the breast.

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Background: Invasive lobular carcinoma (ILC) represents 7-10% of all breast cancer. The prognosis of ILC when compared with invasive ductal carcinoma (IDC) has not consistently been shown to differ. This may be, in part, due to the relatively small number of patients with ILC and its varying histologic criteria for diagnosis.

Materal and Methods: From 1979 through 2001, we treated 2511 patients with invasive breast cancer. Clinical, pathologic, histologic, and survival data for both IDC and ILC were compared.

Results: Aside from the fact that ILC were bigger at diagnosis, all other prognostic factors were more favorable. This resulted in a better distant disease free survival (DDFS) and breast cancer specific survival (BCSS). Unlike lobular carcinoma in situ, in which bilaterality is common, the percentage of contralateral breast cancer was similar for patients with either IDC or ILC.

Discussion: When corrected for size, patients with ILC have a better prognosis than those with IDC. All prognostic factors studied in this series were generally more favorable for patients with ILC.

	Ductal	Lobular	P
Number of Patients	2209 (88%)	302 (12%)	
Average Age	53	56	0.007
Average Tumor Size	24 mm	41 mm	0.00001
% Nonpalpable	23%	23%	NS
ER Positive	71%	84%	0.0001
PR Positive	62%	73%	0.002
Average Nuclear Grade	2.32	1.83	0.00001
% Diploid	46%	63%	0.007
% S-Phase > 6.0	48%	33%	0.004
Percent Positive Nodes	36%	32%	NS
% Her2/neu Overexpression	29%	7%	0.00001
10-year DDFS	74%	77%	0.05
10-year BCSS	78%	84%	0.04
Contralateral Breast CA	6.8%	6.7%	NS

123 Health related quality of life (HRQOL) and psychosocial status at diagnosis are not associated with disease-free or overall survival (DFS or OS) in T1-3, N0-1, M0 breast cancer (BC).

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Background: Psychosocial status and HRQOL have been postulated to influence outcomes in early stage BC, however existing evidence is weak. We examined the prognostic associations of these factors in a prospective cohort study.

Methods: 378 women with surgically resected T1-3, N0-1, M0 BC, enrolled at 3 University of Toronto centers between 1992 and 1996, completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (Core 30 items) (EORTC QLQ-C30), the Profile of Mood States (POMS), the Psychosocial Adjustment to Illness Scale (PAIS), the Impact of Events Scale (IES), the Mental Adjustment to Cancer Scale (MAC) and the Courtauld Emotional Control Scale (CECS) 2 months post diagnosis. Data on tumor-related factors, treatment and outcomes were obtained prospectively from medical records, and Cox survival analyses performed after 57 distant recurrences and 34 deaths (median follow-up 5.8 years).

Results: Mean age was 52.5±9.9 years. 218 women had T1, 127 T2, 14 T3 and 19 TX tumors; 117 were N1. 100 received adjuvant chemotherapy, 127 hormone therapy, 44 both and 107 neither. A greater impact of BC and its treatment on vocational environment (PAIS) was univariately associated with shorter DFS and OS (p=0.003, p=0.02); these associations were non-significant after adjustment for age, BMI, tumor and nodal stage, and adjuvant therapy (p=0.06, 0.41). A similar pattern was present for role functioning (EORTC QLQ-C30), p=0.047 unadjusted, p=0.24 adjusted. Anger (CECS) had a borderline association with OS that was significant in adjusted analyses (p=0.17 unadjusted, p=0.048 adjusted) but not DFS (p=0.66 unadjusted, p=0.19 adjusted). No significant associations with DFS or OS were seen for global QOL (EORTC) (p=0.72 DFS, p=0.29 OS), for other EORTC QOL subscales, for POMS (total or subscales), IES (total or subscales), MAC (all subscales) or other PAIS or CECS scales.

Conclusions: In general, HRQOL and psychosocial status at diagnosis are not associated with distant recurrence or survival in BC.

124 Prognostic value of HER2 overexpression in metastatic breast cancer patients treated with standard dose chemotherapy +/- high-dose consolidation treatment.

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Background: HER2 is overexperessed in 25-30% of human breast cancer. Clinical data suggest that the overexpression of HER2 is a poor prognostic factor in metastatic breast cancer (MBC) patients (pts) treated with hormonotherapy or chemotherapy. Pts with overexpression of HER2 receiving anthracycline/paclitaxel-containing regimens have a better outcome. Material and Methods: We have analyzed data from 88 pts with MBC. Expression of HER2 was evaluated by IHC method (Herceptest, DAKO). Chemotherapy regimens (SCT) included Epirubicin + Paclitaxel +/ - Gemcitabine. Forty-two pts (47.7%) received consolidation with high-dose chemotherapy (HDC). Median age was 49 years (range 25-71). HER2 was overexpressed (score 3+) in 25 pts (28.4%). After treatment 29 pts (33%) were in CR, 41 pts (46.6%) in PR 16 pts (18.2%) with SD. In the group with HER2 3+ the CR, PR and SD rates were 40%, 36% and 16% respectively vs. 30.2%, 50.8% and 19% of the pts with HER2 0-2+. The correlation between HER2 status and response was not statistically significant. Median (95% C.I.) PFS and OS were 13.4 months (mos) (11.1-15.7) and 33.7 mos (19-48.5) respectively. Median PFS of pts with or without HER2 overexpression was 12.5 mos (8.6-16.5) and 13.5 mos (11.1-15.9) respectively. Median OS of pts with or without HER2 overexpression was 39.1 mos and 28.9 mos (16-41.9) respectively. The differences were not statistically significant. Median PFS of pts treated with SCT was 12.6 mos (9.8-15.3) vs. 10.8 mos (9.6-12) for HER2 3+ and HER2 0-2+ respectively (p.0.7). Median OS was 26.1 mos (19.6-32.5) for HER2 0-2+ and it has not been reached yet for HER3 +: p: 0.06. Median PFS after HDCT was 13.6 mos (8.5-18.8) and 19 mos (14.2-23.8) for HER2 3+ and HER2 0-2+ respectively: p: 0.03. Median OS was 21.5 mos (7.5-35.5) and 48.5 mos (28-69) for HER2 3+ and HER2 0-2+ respectively: p: 0.1. Our data suggest that HER2 status is not a predictive factor for response. Moreover the correlation of HER2 status with PFS or OS after SCT was not significant. Our data suggest that HER2 overexpression is a poor prognostic factor on PFS for patients treated with HDC.

125 The significance of HER-2/neu as prognostic factor in primary breast cancer depends on the parameters included in the multivariate analysis.

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Purpose: Many of the more than 180 prognostic factors for primary breast cancer are significant for OS (overall survival > 5 years) in univariate analysis, but not in multivariate analysis. The purpose of this study was to analyse systematically these discrepancies taking as example c-erB-2/HER-2/neu.
Patients and Methods: We monitored 471 pts patients with primary breast cancer for a median of 62 months (median age 43.7 y, T1 (29%), T2(58%), N0(46%), grade 1 (28%), grade 3 (32%), ER (0-19%; 28%), ER (>50%, 19%), HER-2/neu (77.3% no overexpression).
Results: The most important parameters with respect to OS were the classical clinical factors nodal status, tumor size and grading: N0 (OS: 78%); N1(OS: 57%); p<0.0001; T1,T2 (OS: 72%); T3,T4 (OS: 31%); p<0.0001; grade 1 (OS: 80%); grade2,3 (OS: 61%); p=0.0006. HER-2/neu as an univariate parameter was significant: No overexpression (=NO)(OS: 69%); overexpression (=O)(OS: 56%); p=0.03. Multivariate analysis revealed that the significance of HER-2/neu depends on the parameters which were included in the multivariate analysis. Taking HER-2/neu and the nodal status, then HER-2/neu is significant as prognostic factor for OS in N1 (O (OS: 40.5%); NO (OS: 62.1%); p=0.0110), but not in N0 (p=0.5546). Taking HER-2/neu and T, than HER-2/neu is neither significant in the subgroup T1,T2 nor in T3,T4. T1,T2 (O (OS: 67.2%); NO (OS: 73.8%); p=0.3144). T3,T4 (O (OS: 21.1%); NO (OS: 36.1%); p=0.2505). The same is true for HER-2/neu and grading. Grade1: O (OS:100%; only 2 pts); NO (OS:80.2%); p=0.3231) and grade2,grade3 (O (OS: 43.4%); NO (OS: 63.7%); p=0.0110). Pts obtaining TAM as adjuvant therapy had no significant difference in OS with respect to HER-2/neu (O (OS: 70.6%); NO (OS: 74.6%); p=0.6473). For pts obtaining chemotherapy HER-2/neu was again significant (O (OS: 45.0%); NO (OS: 65.1%); p=0.0186).
Conclusion: Whether HER-2/neu is significant or not i.e. gives additional information for OS, depends on the parameters included in the multivariate model. This may be one explanation for the various contradicting results with respect to this parameter.

126 Prospective evaluation of tumor markers (c-erbB-2 oncoprotein, CEA and CA 15.3) in patients with locoregional breast cancer.

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Tumor markers were prospectively studied in the sera of 496 untreated patients with breast cancer diagnosed between 1988 and 2001. Abnormal c-erbB-2 levels (>15 U/ml) were found in 7%, CEA(>5 ng/ml) in 12% and CA 15.3 (> 30 U/ml) in 13% of the 496 patients. C-erbB-2 serum levels were only related to c-erbB-2 in tissue, with significantly higher concentrations in patients with positivity in tissue. All tumor markers (c-erbB-2 only in patients with positivity in tissue) were correlated with tumor size, TNM and nodal involvement. CEA was also related to menopausal status, c-erbB-2 and ER. Univariate analysis (mean follow-up 8 years) show that CEA, CA 15.3 and c-erbB-2 were prognostic factors with significantly shorter disease free survival (DFS) and overall survival (OS) in patients with pretreatment tumor marker positivity. Multivariate analysis (445 patients) in DFS and in OS show that nodal involvement, CEA and ER but not Tumor size, menopausal status, histological grade, histology, CA 15.3, c-erbB-2, PgR, adjuvant treatment, p53 (345 patients) or c-erbB-2 in tissue are independent prognostic factors. Multivariate analysis in node positive and in node negative patients show that tumor size, ER, menopausal status and CA 15.3 (node negative) are independent prognostic factors. In summary, tumor markers are an useful, cheap and reproductible tool in prognosis.

127 Invasion factors uPA/PAI-1 and HER2 status provide independent and complementary information on patient outcome in node-negative breast cancer.

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Purpose: The clinical relevance of invasion factors uPA / PAI-1 and HER2 status was evaluated in node-negative breast cancer patients (n=118) without adjuvant systemic therapy after long-term follow-up of more than 10 years (median 126 months).
Patients and methods: Levels of uPA (urokinase-type plasminogen activator) and its inhibitor PAI-1 were prospectively measured by ELISA in primary tumor tissue extracts. HER2 gene amplification (HER2_AMP) was evaluated by fluorescence in situ hybridization (FISH) (Ventana Medical Systems HER-2/neu probe) and HER2 protein overexpression (HER2_EXP) by immunohistochemistry (Oncogene Science antibody Ab-3) on parallel-cut formalin-fixed paraffin-embedded tissue sections.
Results: uPA/PAI-1 was high (either or both factors high) in 44 % of the tumors. HER2 gene amplification was detected by FISH in 33 % and HER2 overexpression was found by IHC in 44 % of the cases. In multivariate analysis of established and tumor biological prognostic factors, uPA/PAI-1 was the only independent prognostic factor for disease-free survival (DFS) (p<0.001; RR 8.3). While HER2_AMP and HER2_EXP did not reach significance for DFS, they were significant for OS, even in multivariate analysis (HER2_AMP: p=0.004; RR 3.7; HER2_EXP: p=0.009; RR 3.4).
Conclusion: After long-term follow-up, uPA/PAI-1 levels in primary tumor tissue reliably and strongly indicate an aggressive course of disease in node-negative breast cancer independent of HER2 status. The particular prognostic impact of HER2 status on OS may reflect its ability to predict resistance to systemic therapy.

128 The prognostic value of HER-2/neu status in premenopausal patients with hormone-responsive breast cancer.

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Background: HER-2/neu status in breast cancer tissue has shown to be a marker for aggressive tumor biology. In the palliative setting, HER-2/neu status is predictive for response to Herceptin® (trastuzumab). Furthermore, several retrospective trials indicate anthracycline sensitivity and tamoxifen resistance. Little is known about HER-2/neu status in premenopausal patients with hormone-responsive tumors.
Patients and Methods: Austrian Breast & Colorectal Cancer Study Group (ABCSG) Trial 5 has shown that combination endocrine treatment with adjuvant tamoxifen (20mg, 5a) and goserelin (3.6mg q 4w, 5a) is superior to i.v. cyclophosphamide, methotrexate and fluorouracil (CMF) in 1040 premenopausal patients with hormone-responsive breast cancer (J Clin Oncol, in press). We investigated whether HER-2/neu status is prognostic and/or predictive for these patients (n=572), or either one of the treatment groups.
Results: Strongly positive HER-2/neu (+++) status was detected in 12.2% of all patients at 5 years, representing a significant predictor of worse overall survival (OS) (92% vs. 82%; p<0.01) and relapse-free survival (77% vs. 72%; p=0.07). No significant interaction was established in analyses of results according to treatment groups. Analyzing results further, interaction showed to be significant between the prognostic value of HER-2/neu status and tumor grading. HER-2/neu status failed to affect OS in the presence of G3 tumors, but was highly significant (p<0.0001) in patients with better-differentiated tumors (95% vs. 82% OS at 5 years).
Conclusions: HER-2/neu status represents an indicator for poorer outcome in this homogeneous patient group. No treatment interaction was demonstrated either for endocrine or cytotoxic treatment. HER-2/neu status shows additional, inferior prognostic significance in patients with G1 and G2 tumors, but not so in G3 tumors.

129 Progesterone receptor quality and level have a significant prognostic and predictive impact in pre- and postmenopausal patients with hormone-responsive breast cancer: six-year results of ABCSG trials 5 and 6.

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Backgound: Proper selection of adjuvant and palliative treatment in breast cancer patients requires hormone receptor measurement in tumor tissue to discriminate between hormone-responsive and -unresponsive tumors. Estrogen receptor (ER) levels are predominantly used to this end. We compared the prognostic and predictive impact of the ER and progesterone receptor (PgR) in ABCSG Trials 5 and 6 participants.

Materials and Methods: 1040 premenopausal (premeno) and 1975 postmenopausal (postmeno) patients with ER- and/or PgR-positive tumors measured by immunocytochemistry were accrued to these adjuvant trials. Premeno women were randomized to CMF (x6) or tamoxifen (5a) and goserelin (3a), and postmeno patients to either tamoxifen (5a) or the same treatment plus aminoglutethimide (2a). After 6 years, endocrine treatment was shown to be significantly superior to CMF in the former (JCO, in press), whereas the difference between the 2 treatment groups was not significant in the latter. ER and PgR positivity was based on the proportion of positively stained tumor cells and intensity of staining, resulting in 4 different levels: -, +, ++, +++ (Reiner et al, Cancer Res 1990).

Results: ER and PgR distribution according to the 4 levels was 7.1, 32, 36.3 and 24.6%, and 10.6, 19.6, 31.3 and 38.3%, resp. In multivariate analyses for overall survival (OS), recurrence-free survival (RFS) and event-free survival (EFS), only PgR showed prognostic significance. Taking the PgR levels into account, patients with PgR +++ tumors experienced significantly improved OS, RFS and EFS than others (e.g., RFS at 6a: PgR - 72, + 77, ++ 80, +++ 82%; p<0.0005). For postmeno women, distribution of ER and PgR levels was 2.4, 21.6, 34.1 and 41.9%, and 18.6, 23.5, 25.6 and 32.3%, resp. Again only PgR showed a significant prognostic impact in multivariate analysis of survival. Discriminating between the 4 different PgR qualities, patients with stronger PgR positivity showed significantly superior OS, RFS and EFS (e.g., EFS at 6a: PgR - 75.3, + 79, ++ 82.2, +++ 85.2%; p<0.0001).

Conclusion: For pre- and postmeno patients with hormone-responsive tumors, PgR quality and level have a significantly higher impact than ER, especially for those treated with endocrine therapy.

130 Critical balance of scaffold attachment factor SAFB levels play important role in breast tumor suppression.

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Background: SAFB is a nuclear protein that has been implicated in a multitude of different processes, including maintenance of nuclear structure, chromatin organization, RNA processing, and repression of estrogen receptor (ER) activity. The ER corepressor activity could at least in part explain its tumor suppressor-like characteristics in human breast cancer - it maps to a locus of extremely high LOH (19p13), and it is a strong growth inhibitor. Previous analysis of SAFB in a "dead-end bank" lacking clinical follow-up information revealed that SAFB expression was inversely correlated with ploidy and S-phase. To explore whether SAFB could play a role as a prognostic factor, we measured its expression in breast cancer specimens with detailed clinical data and follow-up

Material and Methods: A western blot study was performed using tumors from axillary lymph node-positive patients, and data were stratified into i) no treatment and ii) endocrine treatment.

Results: In the treatment group we detected a significant correlation of SAFB expression with aneuploidy. There was no correlation with overall survival (OS), but there was a trend towards a shorter disease free survival (DFS) in patients with low SAFB levels. This correlation was not detected in the "no treatment group". However, patients with low SAFB had a significantly shorter OS (p=0.049) as compared to patients with medium and high levels of SAFB. Low SAFB levels remained significantly adverse in a multivariate analysis (Hazard ratio (HR)=1.83, p-value= 0.0245), along with nodes>3 (HR=1.99, p-value=0.013) and low ER expression (HR= 2.2, p-value= 0.0064).

Discussion: As expected patients with low SAFB had a worse OS. Paradoxically, we also detected a high frequency of aneuploidy in tumors overexpressing SAFB. While this might be attributed to different tumor characteristics in the different groups, we will also discuss the possibility that disturbance of balance, either by gain or loss can be detrimental for a cell.

131 Correlation between cyclin D1 vs prognosis and nodal involvement in breast cancer patients: a quantitative study.

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Background:Cyclin D1 is part of the family of cyclins, essential for the G1 phase progression of the cell cycle, by activating it from its quiescent G0 state. It has been implicated in the pathogenesis of many cancer cell types, including breast carcinomas. 45-50% of primary ductal carcinomas overexpress this oncoprotein. This study aims to quantify the level of mRNA expression in patients with breast cancer and correlate this information with both nodal involvement and prognosis of these patients.

Methods:113 patients with varying stages of breast cancer were included in the study. Tissue samples were obtained from these patients and their mRNA extracted, converted to cDNA and subjected to quantitative PCR analysis. Background levels of cyclin D1 from 32 normal breast samples were obtained and analysed in a similar manner.

Results:The mean number of copies of cyclin D1 mRNA in 50 ng of normal breast tissue is 43.05 compared to 53.91 copies in breast cancer tissues, p<0.001. Patients with a poor clinical prognosis, i.e.local or distant metastasis, and/or died of breast cancer, have significantly higher copy numbers of cyclin D1 mRNA than those have a good clinical outcome from treatment, 57.80 versus 49.98 copies (p=0.02). The mean time of follow-up was 72 months. 104 patients were involved in this segment of the study, as 9 patients had died of other causes during the follow-up period. Cyclin D1 may also be associated with nodal involvement. Using the Nottingham Prognostic Index (NPI) as a reference, we showed that patients with a good NPI prognosis, i.e. NPI-1 have lower copy numbers than those with NPI-2 or NPI-3 scores. The values were 47.16, 64.72 and 55.79 copies respectively; p<0.001 NPI-1 vs. NPI-2 and p<0.0001 NPI-1 vs. NPI-3. We found no significant difference in the copy numbers of cyclin D1 when comparing the different stages of the disease. All statistical analysis in this study was performed using a 2-tail Student t-test.

Conclusions:We have been able to quantify the level mRNA expression of cyclin D1 in both normal breast tissues and those with breast cancer. We have also shown that an increased number of cyclin D1 mRNA copies is associated with both nodal involvement and a poorer eventual outcome. These results confirm the role of cyclin D1 as a indicator of poor outcome in breast cancer.

132 PTEN and Cyclin D1 status and outcome in high risk primary breast cancer (HRPBC) patients (PTS) treated with high-dose chemotherapy and stem cell support (HDC).

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Background: Cyclin D1 plays a key role in cell transition from G1 to S phase. PTEN downregulates IP3, affecting signal transduction. Data correlating disease-free (DFS) and overall survival (OS) with PTEN and Cyclin D1 status are mixed.

Materials and Methods: Paraffin-embedded tumor blocks were obtained on 96 women with HRPBC (stage II with ≥ 10 positive lymph nodes, or stage III) who received cyclophosphamide, thiotepa, carboplatin (CTCb) consolidation after an anthracycline-containing chemotherapy induction between 1991-2001. Immunohistochemistry (IHC) for PTEN (Ab-2 antibody, Neomarkers) compared cancer to adjacent epithelial cells, classifying PTEN as absent or markedly reduced vs having normal expression. Cyclin D1 (5D4 monoclonal antibody, Immunotec) was considered overexpressed if staining was detected in >5% of cells. Median follow-up is 39 months (range 6-120 mos).

Results: PTEN expression was reduced or absent in 32% of cases. Cyclin D1 was overexpressed in 65% of cases. The DFS for pts with a reduced/absent and normal PTEN was 48%, 58%, respectively (p=0.30). OS for pts with reduced/absent and normal PTEN was 69%, 80%, respectively (p= 0.21). DFS for pts with overexpressed and absent Cyclin D1 was 57%, 60%, respectively (p= 0.59). OS for pts with overexpressed and absent Cyclin D1 was 84%, 62% respectively (p=0.06). Cyclin D1 overexpression correlated with ER/PR positivity (p<0.01).

Conclusion: The borderline significance of Cyclin D1 suggests that this should be further explored in a larger sample size to ascertain whether it identifies pts who have improved survival after high dose alkylator therapy.

133 Expression of basal cytokeratins in estrogen and progesterone receptor negative, high grade infiltrating breast carcinomas. Is there any prognostic significance?

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Background: Evaluation of breast carcinomas using a variety of cDNA microarrays have shown a distinct subset of estrogen receptor (ER) negative tumors that express basal cytokeratins (CK). It has been suggested that this subset has a significantly worse prognosis than ER negative carcinomas without basal cytokeratin expression. The purpose of our study was to retrospectively assess basal CK expression in estrogen and progesterone receptor (PR) negative high grade infiltrating ductal carcinomas and determine if basal CK expression correlated with survival.

Material and Methods: We searched the archives of Rush Presbyterian-St. Luke's Medical Center for women diagnosed with ER negative, PR negative, high grade infiltrating ductal carcinoma between 1975 and 1988. The cases were divided into two distinct groups that were matched for age, clinical stage, tumor size and lymph node status. Group I consisted of 16 women who were alive and free of disease with a mean of 14 years, and group II consisted of 20 women who had died within a mean of 3 years of diagnosis. Immunohistochemistry for basal cytokeratin (CK5/6, Dako, Carpinteria, CA) and low molecular weight cytokeratin (CK 8/18, Dako, Carpinteria, CA) was performed on formalin fixed paraffin embedded sections.

Results: In group I, 25% co-expressed CK8/18 and CK5/6, 31% cases expressed only CK8/18, 13% expressed only CK5/6, and 31% did not express either. In group II, 35% co-expressed CK 8/18 and CK5/6, 45% expressed only CK8/18, 20% expressed neither, and no cases expressed only CK5/6. Cases showing no immunostaining with either CK5/6 or CK8/18 were thought to have lost antigenicity and were removed from further analysis. Thus, 6 of 11 (54.5%) tumors from group I and 7 of 16 (43.7%) tumors from group II showed some CK 5/6 staining.

Discussion: While the number of patients is small, the tissue expression of CK 5/6 in the primary breast carcinoma was not a negative predictor of survival. Further studies should be performed to clarify the significance of the expression of basal cytokeratins in breast carcinoma.

134 Presentation of 50 phyllodes tumor cases: multivariate analysis of recurrence and mortality predictive factors.

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Background:The phyllodes tumor is a rather infrequent neoplasia, representing 3% of breast pathology. The macroscopic and histological aspect has caused controversy in the biological behavior. There are no predictive factors to certainly determine evolution and prognosis of this neoplasia. This investigation presents the most constant clinical and histopathological factors of recurrence and mortality.

Material and Methods: Fifty files of patients with histologic diagnosis of Phyllodes Tumor from the Breast Tumor Oncology Service were reviewed. Age, Evolution time, clinic diagnosis, disease free interval, treatment given, and survival were assessed. Pathological slides of each patient were examined in order to validate: tumor size, edges and growth features, cellular atypia, stromal overgrowth, mitotic index, cellularity, and surgical edges. A multivariate analysis, square chi, exact Fisher's test, variance analysis and a patient survival curve is carried out.

Results: The median age of the patients was 44.9 years. In 16 patients was performed mastectomy and wide excision in 34 patients. Fourteen patients (28%) developed local recurrence. Three patients (6%) developed lung metastases and died. The follow-up had a median of 56.1 months. The multivariate analysis show that the margins, surgical limits, the hipercellularity, the stromal overgrowth, the cellular atypia and the type of surgery were statistically significant for recurrence. The Kaplan-Meier analysis show that the surgical limits, the type of surgery, the hipercellularity, the cellular atypia and stromal overgrowth were statistically significant as predictive factors both in the multivariate and univariate analysis and both in the Kaplan-Meier analysis. Conclusions: The biological behavior of the phyllodes tumor is unpredictable, in this analysis the affected surgical borders, stromal overgrowth, presence of greater number of atypias and the mitotic index are reliable predictive factors for the disease's recurrence which is directly related with patient's mortality.

135 The prognostic value of the axillary fluid in patients with breast cancer.

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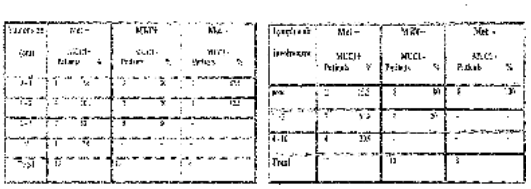
Background: Drains are inserted in the dissected axilla of most patients who undergo operations for breast cancer. The purpose of this study was to evaluate the presence and the prognostic value of MUC1 and Met-HGF/SF in the axillary drainage of these patients.

Methods: Thirty-one consecutive patients with invasive ductal carcinoma of the breast, who were suitable for breast conserving treatment, were studied. The output of the drains, which had been placed in the axilla during operation, was collected, and the presence of MUC1, Met-HGF/SF and β actin were assessed in the lymphatic fluid, by RT-PCR assays. The data were compared to the pathological features of the tumor, the axillary lymph nodes status, immunohistochemical staining and to the estrogen and progesterone receptors status.

Results: RT-PCR assays of the axillary lymphatic drainage were positive for MUC1 and Met-HGF/SF in 13 (41.9%) and 23 (74.2%) of the patients, respectively. Patients in whom MUC1 and Met-HGF/SF were not found in the axillary fluid had smaller tumors, and less capillary and lymphatic invasion, compared to patients with positive assays ($P<0.02$, for all these comparisons). The lymph nodes were negative for metastases in all of the patients with negative assays ($P<0.001$). The presence of MUC1 and Met-HGF/SF showed negative correlations with the estrogen and progesterone receptors ($p<0.05$).

Conclusion: MUC1 and Met-HGF/SF can be detected in the axillary fluids of patients with breast cancer. The expression of both tumor markers in the axillary drainage is strongly associated with unfavorable tumor features, and can be used as a prognostic factor.



136 Analysis of incidence and prognostic factors for local recurrence and its impact on survival of women with node-negative breast cancer.

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A consecutive series of women with node-negative breast cancer treated at eight Toronto hospitals between 1987 and 1998 has been followed since that time. The objectives of this study are: 1) to establish the incidence of local (within breast) recurrence in this cohort, 2) to evaluate factors prognostic for local recurrence, including neu/erbB-2 status and 3) to assess the impact of local recurrence on disease-free survival. The adverse impact of neu/erbB-2 status on disease-free survival in 580 women has been reported previously. Women between the ages of 18 and 75 years were invited to participate by their treating surgeon. Eligibility criteria included resection of a primary breast cancer with clear margins and an axillary node dissection with at least 4 sampled nodes. Pathological features including tumor size, histologic and nuclear grade, endothelial space invasion, multicentricity, estrogen and progesterone receptor status, and presence of carcinoma in situ were recorded. DNA analysis (predominantly by quantitative polymerase chain reaction) of tumor samples for neu/erbB-2 amplification was performed where available. Treatment and followup data were collected in a standardized fashion without knowledge of the molecular analyses. A preliminary analysis involving 1005 women has been performed. A total of 82 (8.2%) women have developed local recurrence; of which 60 developed an isolated local recurrence and 22 developed both local and distant recurrence. 118 (11.7%) women developed distant disease without local recurrence. At the meeting we will present updated data on over 1500 women, with follow-up complete at June 2002. Multivariate analysis will be performed investigating the effect of traditional factors (age, menopausal status, tumor size, tumor grade, presence of in-situ disease, multicentricity, hormone receptor status) and neu/erbB-2 status on local recurrence. The influence of local recurrence on subsequent outcomes including disease-free survival will be presented.

137 How young is too young? The impact of age on premenopausal breast cancer prognosis.

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Background: The definition of "young age" as an adverse prognostic factor for breast cancer (BC) is unclear. This study examines the impact of age on BC outcomes.

Materials and Methods: Data from 4286 women aged <50, diagnosed with invasive BC between 1989-1998, were abstracted from the British Columbia Cancer Agency's Breast Cancer Outcomes Database, and analyzed according to 5 age categories: <30 (N=92), 30-34 (N=288), 35-39 (N=688), 40-44 (N=1392) and 45-49 (N=1826, control).

Results: Median follow-up was 7.3 years. Women aged <40 presented with more stage III-IV cancers (18% vs. 14%, p<0.0001) and had more grade III (p<0.0001), ER-negative (p<0.0001) and lymphovascular invasion positive tumors (p<0.0001), compared to women aged >40 . Although mastectomy (41%) and radiotherapy (69%) rates were similar, women aged <40 received more chemotherapy (53% vs. 46%, p<0.0001) and less hormone therapy (22% vs. 26%, p=0.003) than women aged >40 . Five-year BCSS, OS and LRFS were significantly worse for women aged <40 with stage I-II disease (table 1). Hazard ratios, determined by multivariable Cox analysis, are displayed in table I.

Discussion: In spite of similar rates of mastectomy and radiotherapy, and higher rates of adjuvant chemotherapy, women aged <40 at diagnosis had significantly worse LRFS, BCSS and OS for stage I-II BC.

Kaplan Meier and Cox Analyses

	Stage	<30	30-34	35-39	40-44	45-49	p-value
5-year Overall Survival	I	.83	.89	.87	.97	.96	0.002
	II	.64	.72	.71	.83	.86	<0.0001
	III	.60	.52	.44	.59	.64	0.09
	IV	.20	.08	.17	.16	.27	0.28
Hazard Ratio OS*	all	1.8	1.7	1.5	1.0	1.0	<0.0001
5-year Breast Cancer-Specific Survival	I	.89	.89	.89	.97	.96	0.0003
	II	.64	.73	.73	.84	.87	<0.0001
	III	.68	.52	.46	.64	.66	0.06
	IV	.20	.11	.20	.21	.29	0.51
Hazard Ratio BCSS*	all	1.8	1.9	1.6	1.1	1.0	<0.0001
5-year Local Relapse-Free Survival	I-II	.90	.84	.88	.93	.95	<0.0001
Hazard Ratio Local Relapse*	I-II	1.1	2.5	1.7	1.2	1.0	0.0006

*multivariable analysis included stage, er-status, grade, lymphovascular invasion, surgical margin status, systemic therapies, type of local surgery, & radiation

138 Clinical outcome of breast cancer patients with liver metastases in the anthracycline-taxane era.

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Introduction: The small population of patients (pts) with metastases limited to liver has been reported to have a 19 month median survival with pre-taxane chemotherapy (CT) regimens (J Clin Oncol 5: 773-82,1987). No recent data has been reported with respect to survival of this patient group in the literature.

Methods: The efficacy of adriamycin (A) vs paclitaxel (T), trial 10923, with crossover at the time of progression and of AT vs AC (cyclophosphamide), trial 10961, given as first line CT in metastatic BC pts have been compared in two recent phase III EORTC trials (n=331 and n=275, respectively) (J Clin Oncol 18: 724-33, 2000, Proc Am Soc Clin Oncol 19:73a, 2000). We analyzed the median survival of BC pts with liver metastases (LM) enrolled in these two trials, considering that their clinical outcome would be indicative of what can be achieved nowadays with our best cytotoxic agents.

Results: The median follow up for trial 10923 and 10961 are 85 and 45 months (mo), respectively. Median age was 54 in trial 10961 and 55 in trial 10923.The number of pts with LM alone (LMA)/LM+other sites (LMO) were 29/131 and 20/95 in trials 10961 and 10923, respectively. The median survival of pts with LMO were 13.9 and 16.6 mo and LMA were 22.4 and 26.4 mo in trials 10923 and 10961 respectively. The patients with LMA had a higher probability of survival at 2 years (50% vs 22% in trial 10923; and 55% vs 29 in trial 10961); their detailed characteristics as well as their first sites of disease progression are being analyzed.

Conclusion: Given the high prevalence of BC, improved detection of LM with more specific imaging techniques (MRI and PET) and encouraging survival achieved in pts who have LMA with currently available anthracycline and/or taxane based regimens (and/or Herceptin), a more aggressive multidisciplinary treatment approach including ablation techniques and/or consolidation of response with biological agents seems worth exploring, in this specific subset, in a controlled fashion.

139 Lipid and apolipoprotein profiles of breast cancer in west Algeria, adjuvant hormone therapy, recurrence, and prognosis.

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To study the effect of tamoxifen on the developpement of lipid and apolipoprotein abnormalities in women breast cancer, we have investigated 103 patients receiving adjuvant hormone therapy (tamoxifène, 20 mg/j pendant 3 ans), and chimiothérapy (FEC 50, 6 cures). The patient population consisted of patients with RE+ RP+ tumors, little differentiated from rank SBR III (35.9%) vs SBR II (54.4 %) vs SBR I (9.7%), with 1 to 3 ganglia invaded (55 %). infiltrating ductal carcinomas are most frequent (84.5 %), followed by the lobular infiltrating carcinoma(12.6 %), two peaks incidence range between 40-49 years and 50-59 years, with respectively 26.1% vs 29.1% of the cases. the rate of survival over 03 years without recurrence is of 73 %, and without metastasis of 71.1 %. all patients had two to threefold increased levels of TG, together with a moderate elevation of TC levels. all patients groups had elevated levels of VLDL cholesterol and slightly decreased levels of HDL cholesterol. The apolipoprotein profile of all patients was characterized by significantly reduced levels of (Apo)A-I and (Apo)A-II and significantly increased levels of ApoCIII, ApoB and Lp(a). regardless to TG levels, all patients had lower ApoA-I/ApoC-III ratios than controls. up to 3 years adjuvant treatment, pateints had slighltly higher ApoA-I and ApoA-II and lower ApoB levels compared to levels befor treatment. patients with vascular desease due to tamoxifen side effect, had higher TC, TG, ApoB, ApoC-II than patients without vascular desease. These results demonstrate that the dyslipoproteinemia with breast cancer is already manifested at the early stages od desease through its abnormal apoliprotein rather than lipid profile.

140 Tubular carcinoma of the breast: is the long term survival assured?

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Background: Pure tubular carcinoma (TC) represents only 2-4% of all invasive breast cancers. Previous studies are small but indicate a particularly favourable prognosis. We reviewed the long term (up to 25 years) survival of patients with TC, to investigate whether this lesion does confer better survival than the control group of commoner grade I invasive ductal carcinomas of no specified type, (NST).

Materials and Methods: Case records from 1975 - 2000 were obtained for all grade I invasive ductal carcinomas treated at Guy's Hospital London. There were 69 patients with TC (>90% tubule formation) and 420 grade I (NST). The mean follow up was 9.7 years. All patients had tumourectomy and level III axillary dissection. Kaplan-Meier survival curves were compiled and statistical analysis was carried out using the Log Rank method.

Results: There was no difference in the mean age of the two histological types of grade 1 carcinoma. Tubular cancers presented with a significantly smaller primary tumour (mean 1.37cm v. 2.12cm, p=0.002). 26.8% of TC were node positive compared to 36.7% of grade 1 NST (not significant p=0.205). Despite the more favourable size and lymph node status of TC no significant statistical differences in long-term survival of patients with TC and those with G1 (NST) were identified (P = 0.339). Moreover the similarity in overall survival remained after sub-division into node negative (p= 0.269) and node positive (p=0.885) groups.

Discussion: Although TC has been thought to be a favourable sub-type of breast cancer, it is concluded that its overall long term survival is no better than that of the commoner grade I invasive carcinoma of non-specific type. Therefore TC should continue to be regarded as potentially life threatening in the longer term and accordingly should not merit an attenuated treatment or follow up protocol.

141 Deprivation and pathological prognostic factors in operable breast cancers.

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Introduction

Patients from deprived areas with breast cancer have a poorer outcome, the reasons for this are unclear. The aim of this study was to assess the influence of deprivation on various pathological prognostic factors in patients with operable breast cancers.

Method

Prospective data were collected on all patients who had operable breast cancers in Glasgow between October 1995 and October 2001. They were separated into three groups - affluent, intermediate and deprived according to the Carstairs index of deprivation at the time of diagnosis. The influence of deprivation on the pathological size of the tumour, ER status, histological grade and axillary node status were examined. The Nottingham Prognostic Index was also calculated for each patient (NPI).

Results

A total of 3251 patients were included, 598 affluent, 1473 intermediate and 1180 deprived. Patients in the deprived group were significantly more likely to have larger tumours with 33.3% of affluent, 35.6% of intermediate and 43.4% of deprived patients having tumours of greater than 20mm ($p<0.001$). There was a higher incidence of patients in the deprived group with high-grade tumours. 27% of affluent, 30% of intermediate and 35% of deprived patients had grade 3 tumours ($p<0.01$). There was also a trend for the more deprived patients to have a higher incidence of ER negative tumours with 18.8%, 21.9% and 24% of patients in the affluent, intermediate and deprived groups having a negative ER status respectively ($p=0.04$). The deprived group were significantly less likely to have a highly positive (>=50%) ER status ($p=0.05$). There was no significant difference in node positivity.

Conclusion

Patients from deprived backgrounds are significantly more likely to have larger tumours at operation. They are also more likely to have high-grade tumours and less favourable ER status. The affluent patients had significantly lower NPIs. This may explain why patients from deprived areas have a poorer outcome.

142 Are some patients cured after local therapy alone? Clues based on natural history data from Turku Finland.

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Introduction: Examining the long term outcome (at least 25 years) of patients who were treated prior to the advent of the widespread use of adjuvant chemotherapy enables us to ask the fundamental question: After surgery and radiation alone, are there some patients who are no longer at risk of disease progression (i.e. cured)?

Methods: 2192 patients with breast cancer from Turku, Finland were meticulously followed in a prospective manner from 1945 to 1996. We examined a subset of 550 patients who were treated with mastectomy plus radiation from 1945-84. No adjuvant therapy was used. Less than 1% were lost to follow-up (FU). The longest FU was 43.08 years, the median and mean 8.3 and 10.7 years, respectively. 293 patients had a recurrence or died of breast cancer. Of the patients who recurred, the last recurrence occurred at 24.7 years. 257 patients either died of something other than their breast cancer, or were last seen still alive- in both cases without any evidence of further disease progression following their surgery and radiation (S&XRT). A special statistical procedure was developed to partion this set of 257 patients into 2 subsets: 1) those patients who were at minimal risk of further disease progression following their S&XRT; and 2) those patients at substantial risk despite their S&XRT. We call this procedure the Minimal Risk Partioning Algorithm (MRPA). MRPA identified 231/257 patients as cured (at minimal risk) and 26/257 as still at substantial risk.

Results: MRPA identified 231/257 or 42% of the patients as cured. This number is very plausible because it is exactly the same as the 42% which was the horizontal asymptote of the original KM analysis. KM curves of the entire population show a decreasing hazard for risk of recurrence over time, however the partitioned curves, in contrast, demonstrate a constant hazard over time. The presence of N1, N2, grade 3, moderate or severe necrosis, M1 features were significant in a step wise Cox Regression analysis. Data on actual numbers of nodes was not available in this data set as the data was recorded according to N stage only. The MRPA allows us to construct tailored curves for each individual based on their risk factors. The curves show, for example, that grade affects not only the likelihood of recurrence but the rate of recurrence as well.

Conclusion: The relatively homogenous genetic composition of the Turku population and the meticulous, lengthy FU make this an ideal data set for assessing the curative impact of surgical intervention alone. The MRPA leads us to believe that 42% of patients were cured without systemic therapy and allows the creation of individually tailored risk assessments, which help us to understand how specific factors affect both the likelihood and rate of recurrence.

143 The influence of prognostic factors seems to be constant throughout the course of metastatic breast cancer regardless of therapeutical intervention.

Ploner F, Genser B, Schippinger W, Hofmann G, Samonigg H. Graz, Styria, Australia

Background: It has been shown in the past that established clinical prognostic factors such as disease-free interval, hormone receptor status, grading and metastatic site at first presentation of recurrent disease predict survival. But the degree of interaction between these factors and the effect of palliative systemic treatment interventions have not been clearly examined.
Patients and Methods: We designed a retrospective study using a historical cohort of patients with metastatic disease to evaluate the impact of prognostic factors throughout the course of metastatic disease and to examine these interactions. 1742 patients diagnosed with breast cancer and 642 of them faced recurrent disease were managed at the Div. of Oncology, Karl-Franzens-Universität Graz between 1989 and 1996. Out of this population we selected a cohort of 270 patients with metastatic disease and 12 months of follow up who had received at least one systemic treatment with evaluation of response documented. Statistical analysis of the clinical data was performed using extended Cox-models, multi-state models and stochastic process modelling.
Results: The analysis revealed significant influence on survival by disease-free interval, hormone receptor status, grading and first metastatic site. The effects of prognostic factors seem to be constant throughout the time course of metastatic disease regardless of systemic treatment interventions.
Discussion: The impact of systemic therapy on survival remains a matter of controversy since in the past decades there weren't performed any randomised trials with a non-treatment arm. But by means of new statistical methods we were able to show that the impact of prognostic factors is constant and independent of systemic intervention keeping in mind that the therapeutical strategy was adaptive and dependent on the course of disease. Future studies are needed to validate these findings and to evaluate other prognostic markers in metastatic breast cancer.

144 Levels of expression of tumor endothelial markers and their correlation with prognosis in patients with breast cancer.

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Background: Tumor endothelial markers (TEM's) are a newly discovered family of endothelial markers associated with tumor specific angiogenesis. This study sought to examine the levels of expression for TEM-1 and TEM-2 in breast cancer tissues.
Material and Methods: 120 frozen breast cancer tissues together with 33 normal background tissues were obtained after surgery. RNA was extracted from frozen sections for gene amplification. The expression of TEM-1 and TEM-2 was assessed using RT-PCR and the quantity of their transcripts was determined using real-time-quantitative PCR (Q-RT-PCR). Statistical analysis was performed using a non-paired Student's t-test.
Results:TEM-1 and TEM-2 were expressed in 71.4% and 35.7% of breast cancer tissues respectively. Using Q-RT-PCR the levels of expression for TEM-1 and TEM-2 were found to be higher in tumor tissues than in normal tissues (0.51 ± 0.78; copies/µg vs. 0.12 ± 0.17; copies/µg), and (199.6 ± 574.2 vs. 97 ± 137.2) respectively. After a median follow-up of 72.2 months it was found that patients with recurrent disease, and/or who had died of breast cancer had a significantly higher TEM-1 level (0.67 ± 1.06) than patients with a good prognosis (0.22 ± 0.30; $P = 0.041$). In addition, patients who had nodal involvement exhibited a significantly higher level of TEM-1 than those who were node negative ($P < 0.01$). Furthermore, the level of TEM-1 and TEM-2 did not correlate with tumor grade.
Conclusion: Breast cancer has raised levels of TEM-1 and TEM-2, compared to normal background tissues. We conclude that levels of TEM-1 are associated with nodal involvement and disease progression, and therefore may have a prognostic value in breast cancer.

145 Shc adapter proteins as prognostic markers in patients with node negative breast cancer.

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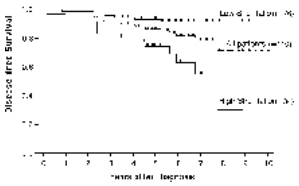
Background: Better prognostic indicators are urgently needed in node-negative breast cancers. Shc signaling proteins are implicated in many pathways associated with aggressive disease. We hypothesized that relatively high amounts of activated, tyrosine phosphorylated (PY) Shc and low levels of an inhibitory, 66-kDa Shc isoform might serve as markers for particularly aggressive cancers.

Methods:Semi-quantitative immunohistochemical (IHC) analyses of PY-Shc and p66 Shc was performed on sections cut from archival blocks of formalin-fixed, paraffin-embedded primary tumor specimens from node-negative breast cancer patients, including patients with ductal carcinoma in situ (DCIS). PY-Shc and p66 Shc staining intensities were then correlated with disease-free survival.

Results: Analysis of primary tumors from an instructive set and a second independent test set of node-negative breast cancers demonstrated that high PY-Shc and low p66-Shc staining correlated with increased risk of recurrent disease. In the combined datasets of 110 patients, 19 of whom experienced recurrent disease(mean follow-up of 6.3 years in non-recurring patients), univariate Cox Proportional Hazards analysis demonstrated the prognostic value of PY-Shc(P=0.025), p66 Shc (P=0.04) and the Shc Ratio (P=0.015,). By multivariate analysis, both PY-Shc and p66 Shc retained high significance (PY-Shc: P=0.007; p66 Shc: P=0.009), and appear independent of tumor size, stage, and hormone-receptor status. Patients with high Shc Ratios (>0.6) had high incidence of recurrent disease (13/34) compared to patients with low Shc Ratios (6/76).



Conclusions: Immunohistochemical measurements of PY-Shc and p66 Shc in primary tumors have excellent prognostic value in node-negative breast cancer, and with further study should guide use and testing of aggressive therapies.

146 The prognostic value of Rho-C and Rho-6 GTPases and Rho guanine nucleotide dissociation inhibitors (GDIs) in human breast cancers.

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Introduction. Rho are small GTPases that regulate malignant transformation and motility of cancer cells. This study determined the levels of expression of Rho-A, B, C and 6 and Rho regulators, guanine nucleotide dissociation inhibitors (GDI)-α, β, & γ in breast cancer.

Methods. Rhos and RhoGDIs were immunohistochemically assessed using frozen sections. The levels of transcripts of these molecules were determined using a real-time quantitative PCR. Levels of expression were analysed against nodal involvement and distant metastasis, grade, and survival over a 6 yr follow-up period.

Results. The levels of Rho-C transcript were significantly higher in breast cancer tissues (133.0±158.5 copies/50ng RNA, n=106) than in background normal tissues (34.4±37.8, p=0.014, n=31). A similar elevation of Rho-6 in breast tumor tissues was also seen. However, the level of Rho-A and Rho-B was found to be similar in tumour and normal tissues. Immunohistochemical staining revealed a stronger staining of Rho-C protein in tumor cells. The levels of Rho-GDIα transcript were found to be significantly lower in tumour tissues (2.5±3.0 copies/50ng) than in normal tissues (26.6±32.6, p<0.001). Rho-GDIγ displayed a similar reduction in breast tissues to that seen with rho-GDIα. The high levels of Rho-C and low amounts of rho-GDI were associated with nodal involvement. In addition, patients with recurrence/metastasis and died of breast cancer had significantly higher levels of Rho-C than those with a good prognosis (176.6±156.7 and 5453.3±9037.9copies/50ng, vs 58.8±63.8, p=0.02 and p<0.001, respectively), after a 6yr follow-up. In contrast, patients with poor prognosis had a significantly lower level of Rho-GDI than those with good prognosis.

Conclusion. Raised levels of Rho-C and Rho-6 and reduced expression of Rho-GDI in tumor tissues are correlated with the nodal involvement and metastasis. The imbalanced expression of Rhos and Rho-GDI is therefore an important prognostic factor in breast cancer.

147 Prognostic role of phosphorylated p38 (P-p38) in node-positive breast cancer.

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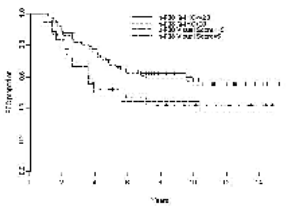
Background: Receptor phosphorylation and dimerization activates intracytoplasmic signal transduction pathways that result in increased cell proliferation, invasion, and metastatic capacity. There are at least three distinct mitogen-activated protein kinase signaling molecules which mediate extracellular signals into the nucleus to turn on the responsive genes in breast cancer cells. These include the extracellular mitogen-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38. Radiation and chemotherapy-induced p38 phosphorylation leads to increased apoptosis in breast cancer cells. P38 is being evaluated as a potential therapeutic target for solid tumors. The purpose of this study was to evaluate the prognostic role of P-p38 expression in human breast cancer.

Material and Methods: We measured P-p38 expression in invasive breast carcinomas and their adjacent normal tissue and ductal carcinoma in situ (DCIS) component. All the patients had node-positive breast cancer and received adjuvant doxorubicin-based chemotherapy. Patients were followed for a median time of 11 years after initial cancer surgery. Immunohistochemistry (IHC) was performed using the peroxidase method and a human anti-P-p38 monoclonal antibody (New England Biolabs). Image analysis was performed on a CAS 200 Image Analyzer (Becton Dickinson). Quantitative results are reported in arbitrary units of optical density corresponding to the staining which is indicative of the amount of antigen on the tissues (Q-IHC). P-p38 expression was also evaluated visually using a 1-9 scoring system.

Results: There was moderate agreement between Q-IHC and visual scores (k=0.55). Although there was a trend between high P-p38 expression and shorter progression-free survival (PFS), the difference was not statistically significant (p=0.39). Larger tumors tended to have higher P-p38 expression. There was no correlation between P-p38 expression and ER/PR status, age, menopause status, nuclear grade or tumor size. High P-p38 expression in invasive tissue was associated with high P-p38 levels in both normal tissue and DCIS.



Conclusion: In this study, P-p38 expression was not a strong prognostic factor in patients with node-positive breast cancer treated with adjuvant chemotherapy. Larger studies are needed to define the prognostic and predictive role of P38 in breast cancer patients.

148 Galectin-9 as a predictive factor for metastasis of breast cancer.

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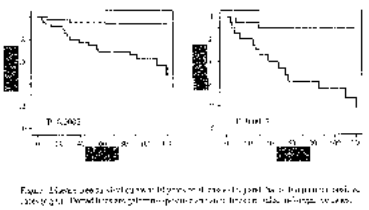
Background: Some of glycoproteins are deeply involved in metastasis of cancer cells. Members of _eta-galactoside-binding galectin family, galectin-1 and -3 are already reported to enhance cancer invasion and protect cancer cells from apoptosis. We now report the functions of galectin-9, a new member of galectins, and relationship between its expression and clinical features in breast cancer patients.

Materials and Methods: To examine the function of galectin-9 in MCF-7 cells, we established galectin-9-overexpressing cells by subcloning and transfection of expression vector of galectin-9, and observed growing styles of cultured cells and transplanted tumors in nude mice. We next examined the expression of galectin-9 in 88 cases of breast cancer patients by immunohistochemistry using anti-galectin-9 polyclonal antibody, and analyzed the correlation between galectin-9 and distant metastasis of breast cancer in comparison with node status.

Results: MCF-7 cells with higher expression of galectin-9 exhibited stronger aggregation in vitro and in vivo. The percentage of galectin-9-positive cases is 50% in breast cancer patients, assessed by HSCORE. Galectin-9 expression reversely correlates to distant metastasis, but neither to lymph node metastasis nor to hormone receptor status. Eighteen cases out of 21 metastatic cases were galectin-9 negative (p=0.0003). Disease-free survival ratio (DFS) of each of galectin-9 positive and negative cases was 93% and 47%, respectively (p=0.0008). Furthermore, DFS of galectin-9 positive and negative cases even in node-positive group was 89% and 19%, respectively (p=0.0017). Multivariate analysis using Cox's proportional-hazards regression model revealed that hazard ratio of galectin-9 expression (13.159) is more than that of node status (5.927), and that galectin-9 is an independent predictive factor for metastasis from other factors.

Discussion: We demonstrate in this paper the tight correlation between cell aggregation and distant metastasis through galectin-9 expression in breast cancer. This suggests the possibility of galectin-9 as a noble and excellent predictive factor for distant metastasis. Galectin-9, but neither galectin-1 nor -3, has a tandem repeat structure in its molecule, which may cause stronger cell aggregation and prevention of distant metastasis. The precise mechanism of galectin-9-induced cell aggregation is under investigation.



149 The prognostic influence of body mass index in premenopausal breast cancer patients.

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Background: The relation of body weight and breast cancer is complex. Higher body mass index (BMI) may be associated with higher incidence of breast cancer in the postmenopause and lower incidence in the premenopause. Little is known about the prognostic impact of current body weight in breast cancer patients. We evaluated the correlation between BMI and outcome of radically resected premenopausal breast cancer patients.

Materials and Methods: BMI was assessed in 417 premenopausal patients in a prospective randomized multicenter ABCSG trial 5 at time of diagnosis and at 3 years postoperatively. The two treatment arms consisted of 6 cycles of chemotherapy (CMF) versus endocrine therapy with goserelin 3.6mg monthly for 3 and tamoxifen 20mg daily for 5 years. Multivariate cox analysis was performed in both treatmentgroups to evaluate the prognostic impact of body weight for overall prognosis of these patients. Changes in body weight were assessed in both therapy groups.

Results: Patients with endocrine treatment as well as those patients receiving adjuvant chemotherapy did show weightgain at the end of adjuvant therapy. Median BMI increased from 24.3 to 25.9 in the endocrine treatment group and from 23.8 to 25.6 respectively, and did not differ significantly from each other. Relative Risk was increased in patients with primarily very low BMI (<18) and very high BMI (>35). Multivariate cox analysis did show a statistical significant influence of squared BMI on overall survival (p=0.0003) and breast cancer related survival (p=0.0014). Impact of BMI on disease free survival was not statistically significant (p=0.08).

Discussion: We could demonstrate for the first time that obese and very slender premenopausal breast cancer patients have a significantly poorer outcome. However, whether or not patients with diagnosed breast cancer should be advised to normalize body weight is an open question. Moderate weight gain after adjuvant therapy was seen in all patients without correlation to the kind of treatment.

150 In patients with primary breast cancer inducible but not endothelial nitric oxide synthase correlates with disease free and overall survival.

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Background: Role of nitric oxide synthase (NOS) in carcinogenesis. Former studies of inducible (i)-NOS and endothelial (e)-NOS in breast cancer patients have revealed controversy results. Previous studies of our group demonstrated on a smaller sample size that patients with NOS positive tumours might have a more favourable prognosis than NOS negative patients (Loibl et al. Cancer 2002 in press).

Material and Methods: 161 patients with primary breast cancer who have had primary surgery in Frankfurt between 1995 and 1998 have been included in our study. We performed immunohistochemical (IHC) staining of paraffin embedded tissue for i-NOS using a polyclonal antibody and e-NOS using a monoclonal antibody. The IHC staining was scored from 0-4, 0 being negative and 1-4 being positive. Correlation with prognostic factors and survival was performed.

Results: The median age at the time of operation was 55 y (31-88y). 39% were premenopausal whereas 61% were postmenopausal. The median tumour size was 2cm; 40.5% of the patients had an involvement of the axillary lymph nodes. 84% of the tumours were hormone receptor positive. 61% of the tumours stained positive for i-NOS and 63% for e-NOS. Expression of e-NOS correlated with the receptor status. Patients with negative receptor status had a significant (p=0.031) higher e-NOS expression than receptor positive patients. There was no difference in the disease free and overall survival rate. Detection of i-NOS was positively correlated with tumour size and with tumour differentiation p=0.03, p=0.006 respectively. In the multivariate analysis i-NOS proved to be an independent prognostic factor. The median time of follow up is 40months, with 55 (34.2%) patients having a local recurrence or metastatic disease. During the observation period of 40months, 7.9% (n=5) of i-NOS negative and 20.4% (n=20) of i-NOS positive patients have died (p=0.033).

Discussion: Significant correlation of i-NOS expression but not of e-NOS expression was found in breast carcinoma with tumour stage, grading and survival and demonstrated i-NOS being an independent prognostic factor.

151 Metaplastic breast carcinoma: a single institution review.

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OBJECTIVE: Metaplastic carcinomas of the breast are rare tumors that are distinguished by the presence of pathologic features of both carcinoma and sarcoma. The goal of this study was to characterize the clinical course, treatment modalities, and clinical outcomes in patients with this neoplasm. METHODS: A retrospective review of the medical records of patients diagnosed with metaplastic breast carcinoma and treated at a university cancer center between 1983 and 1996 was performed. Information was collected on patient demographics, clinical presentation, diagnostic workup, medical and surgical treatment, pathology, and outcome. Survival was calculated by the Kaplan-Meier method. RESULTS: A total of 48 patients were identified with a median age of 47 years at presentation. The median follow up period was 3.1 years. All patients presented with a palpable mass. The clinical stage on presentation was as follows: 5 (10%) patients with stage I, 26 (54%) patients with stage II, 14 (29%) patients with stage III, and 3 (6%) patients with stage IV disease. Estrogen and progesterone receptors were positive in 5 (10%) and 6 (13%) tumors, respectively. Thirty (62%) patients with a median tumor size of 3.0 cm presented with node negative disease on pathology (p<0.001). Six patients received neoadjuvant chemotherapy, 23 patients had adjuvant chemotherapy, and 9 patients received both. Twenty-three (48%) patients received radiation therapy. Fourteen (29%) patients developed local-regional recurrence. No significant correlation was observed between local-regional recurrence and clinical size, type of surgery, nodal status, margin status, or radiation therapy. Twenty-three (48%) patients developed distant metastasis. Presence of metastasis significantly correlated with nodal status (p=0.007) and clinical size (p=0.049). Positive nodal status significantly correlated with survival (p=0.011). The overall five-year survival was 100% for stage I, 50% for stage II, 29% for stage III, and 0% for stage IV (p=0.038). CONCLUSION: Patients with metaplastic breast carcinoma appear to have a higher chance of local recurrence and distant metastasis, as well as a poor prognosis, compared to invasive ductal adenocarcinomas. Therefore, patients with metaplastic breast carcinoma should be treated with surgery and adjuvant therapy.

152 Primary endpoint analysis of the Geparduo-study - preoperative chemotherapy (PCT) comparing dose-dense versus sequential adriamycin/docetaxel combination in operable breast cancer (T2-3, N0-2,M0).

Jackisch C, Von Minckwitz G, Raab G, Schuette M, Blohmer JU, Hilfrich J, Gerber B, Costa S, Merkle E, Eidtmann H, Lampe D, DuBois A, Tulusan AH, Caputo A, Kaufmann M. University, Muenster, Germany; University, Frankfurt, Germany; Red Cross Hospital, Munich, Germany; Bethesda Hospital, Essen, Germany; University Charite, Berlin, Germany; Henriettenstift, Hannover, Germany; University, Rostock, Germany; Markus Hospital, Frankfurt, Germany; Womens Hospital Berg, Stuttgart, Germany; University, Kiel, Germany; University, Halle, Germany; HSK Clinic, Wiesbaden, Germany; Womens Hospital, Bayreuth, Germany; University, Freiburg, Germany; University, Frankfurt, Germany

Introduction: Using a dose-dense PCT (ADOC: adriamycin 50mg/m2 + docetaxel 75mg/m2 q14dx4 + G-CSF) a pathological complete response (pCR) of 9.7% was reported (v. Minckwitz, J Clin Oncol, 2001).

Material and Methods: For the GEPARDUO trial 1000 patients (P) were calculated to show equivalence between this 8 week schedule and a sequential 24 week schedule (AC-DOC: adriamycin 60mg/m2 + cyclophosphamide 600mg/m2 q21dx4 followed by docetaxel 100mg/m2 q21dx4), identical to NSABP B-27. Tamoxifen (20mg/d for 5 years) was given simultaneously in ER/PR positive P. Primary objective was pCR (invasive + in situ) rate within the breast and axilla. Secondary endpoints were toxicity, tumor response by palpation (cRR), and breast conservation (BCT).

Results: The trial was closed prematurely after recruitment of 913 P (458 AC-DOC, 455 ADOC) within 28 months due to striking differences in pCR (Jackisch, SABCS 2001;#509). Median tumour size was 40mm, 60% were N0, clinically. No invasive residual tumor cells were found in the breast 22.4% (AC-DOC) vs. 11.5% (ddAT), pCR in the breast and axilla was 14.1% (AC-DOC) vs. 7.1% (ddAT); p<0.01. For AC-DOC pN0 was 60.7% vs. 55.4% for ddAT. BCT was 74.9% (AC-DOC) vs. 65.5% (ddAT). For AC-DOC cRR was 86% vs 78.2% for ddAT. AC-DOC was associated with a higher rate of grade III/IV nausea (12.7% vs 10.1%), skin/nail toxicity (10.7% vs 3.6%) and neurotoxicity (3.1% vs 1.1%). Hematologic toxicity was reported for neutropenia (66.7% vs 44.7 %), infections (2.9% vs 4.7%); anemia (3.3 vs. 2.5); thrombopenia (1,8% vs. 0%).

Conclusion: Both PCT regimen are feasible and safe. Sequential PCT is superior to dose-dense PCT regarding pCR (breast+axilla), pN0, cRR, and BCT rates.

153 Scarff-Bloom-Richardson (SBR) grading: from predictive to prognostic significance in invasive ductal breast carcinomas treated by neoadjuvant chemotherapy (CT).

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Background: The SBR grade, an important prognostic factor in breast cancer, is also associated with cell proliferation, a consistent indicator of response to CT. The purpose of this retrospective study was to evaluate the influence of SBR grade on response and survival in patients (pts) with operable breast cancer treated by induction CT. Design: the study is centered on 451 pts registered onto 4 prospective phase II trials (from 1982 to 2000). SBR grade according to Elston and Ellis, was evaluated on core needle biopsies collected prior to treatment from 290 pts (pre CT grade) and when possible from residual tumor at surgery (post CT grade). Clinical response was evaluated in 425, and pathological response in 396. Overall survival (OS) and disease free survival (DFS) were calculated by Kaplan Meier Method. Multivariate analysis was used to identify factors significantly related to response and survival.

Results: SBR grade changes were: grade I (14.4% pre CT to 25.5% post CT), grade II (49.0% to 53.3%) and grade III (36.6% to 21.7%). SBR grade III tumors responded better to neoadjuvant CT than SBR grade I ($p<10-6$). None of the other parameters tested correlated with response. None of the other pts and tumour characteristics tested correlated with response. Multivariate analysis revealed that only post CT SBR grade was an independent prognostic factor ($p=2.10-3$ for OS and 8.10-4for DFS). whatever the regimen used ($p < 4.10-2$). Whereas pre CT SBR grade had no influence on prognosis. Moreover, tumour responsiveness was significantly related to changes of the SBR grade ($p = 7.10-3$). Interestingly, down-grading and stabilisation of SBR grade were significantly related with a better OS, compared with up-grading.

Conclusion: SBR grade is a strong predictive factor of response to induction CT in breast cancer, independently of the type of regimen used. SBR grading before and after treatment must be distinguished, considering that pre CT SBR grade is predictive and post CT grade becomes prognostic. This information may prove valuable for clinicians as they make their decision regarding pts therapy.

154 Dose and time intensified epirubicin / cyclophosphamide (EC) as preoperative treatment in locally advanced breast cancer.

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Objective: This study was designed to investigate the effects of primary high dose chemotherapy with dose-intensified Epirubicin (E) (120mg/m^2) and Cyclophosphamid (C) (600mg/m^2) and whether dose-density can have a higher impact on local control and survival.

Method: 262 randomized patients (pts) with breast cancer primarily not suitable for breast conserving therapy (BCT) were treated with EC between 1997 and 2002 in this German multicentered study (T2-T4/ N0-N2/ M0). Eligible for this evaluation were 151 randomized pts. 76 pts received dose and time intensified EC q14 (arm A) and 75 pts dose intensified EC q21 (arm B). G-CFS was prophylactically applied in arm A. After the 3rd cycle surgery was performed. The following adjuvant therapy depended on clinical and histological response. In case of CR/ PR response a 4th cycle of EC was applied followed by 2 cycles of CMF. In NC/ PD response the therapy was changed to a regimen containing Paclitaxel (175mg/m^2, q4) and 5'- FU (2g/m^2, q12). In all pts irradiation after BCT was obligatory.

Results: The overall clinical RR (CR + PR) is 80,8% (arm A 78,9%/arm B 82,6%). The overall pathological RR is 79,8%. pCR was significantly higher in arm B (9,5%) than in arm A (3,9%), ($p=0,04$). There was a downstaging effect on the tumor size as well as on the axillary lymph nodes in more than 75%. After PST BCT was possible in 122pts (80,7%)(arm A 81,5%/ arm B 80%), in22 cases (14,6%) combined with oncoplastic surgery. After a median follow-up of 26 months only 4 pts (2,6%) suffered a local relapse, 10 (6,6%) had metastases and 5 pts(3,3%) died.

Conclusion: Dose intensified EC chemotherapy is a highly effective regimen in the preoperative treatment of locally advanced breast cancer. This treatment has a higher pCR rate compared to the dose and time intensified EC arm. Downstaging is possible in more than 75% in both arms. BCT could be performed in 80,8%. No significant differences in clinical response or the frequency of BCT were observed in either arm.

155 Is there an alternative to anthracycline based chemotherapy: an evaluation of a non-anthracycline containing regimen as neoadjuvant therapy in locally advanced and inflammatory breast cancer.

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Historically most neoadjuvant regimens were anthracycline based. With the development of Herceptin and the discovery of its cardiotoxicity the search for alternative regimens that are equivalent to or better than anthracyline based regimens began. pCR following neoadjuvant treatment is a useful surrogate marker of survival. Fifty seven patients with locally advanced and inflammatory breast cancer were treated with a non anthracycline containing neoadjuvant regimen with evaluation of the pathologic complete response (pCR) as the endpoint.

REGIMEN:Cisplatin 70 mg/m and Docetaxel 70 mg/m were administered Q 21 days x 4 cycles with G-CSF prophylaxis. Patients underwent surgery then adjuvant AC and XRT. Tamoxifen 20 mg/d x 5 years was given to all ER+ pts at the beginning of XRT.

TUMOR CHARACTERISTICS: mean tumor diameter 10 cms. inflammatory 26% (15/57), T4 50% (29/57), T3 50% (28/57), N0 26% (15/57), N1 51% (29/57), N2 23% (13/57). Stage: 2B 17%, IIIA 33%, IIIB 48%, IV 2%. ER+ 53%. Her-2+ 26%

PATIENT CHARCTERISTICS: mean age 49 (range 27-66), premenopausal 53%.Black 39%, Latin 58%, Caribbean Islander 19%.

RESULTS: Clinical response-RR(CR+PR) 96%, No response 4%-One pt with clinical CR refused surgery and is alive and disease free 29 mos later. Pathologic response: pCR in breast 27% (15/56), Node negative 28% (16/56), 1-3 nodes 32% (18/56), 4-9 nodes 21% (12/56),≥ 10 nodes 18% (10/56). pCR in breast and axilla 20% (11/56).

TOXICITY: Grade III/IV: Anemia 4%, hyperglycemia 8%, hyponatremia 2%, catheter infection 2%. One pt died during adjuvant therapy at an outside hospital.

CONCLUSION: The combination of docetaxel and cisplatin is very active in the neoadjuvant setting and delivers pCR rates equivalent to or better than standard anthracycline containing regimens.

156 In-vivo-chemosensitivity adapted preoperative chemotherapy (PCT) in patients (P) with primary operable breast cancer. First experiences of the pilot GEPARTRIO - study.

Von Minckwitz G, Blohmer JU, Du Bois A, Gerber B, Raab G, Eidtmann H, Hilfrich J, Merkle E, Jackisch C, Costa SD, Schütte M, Kaufmann M, for the GABG. University Hospital, Frankfurt, Germany; University Hospital, Berlin, Germany; Horst-Schmidt-Kliniken, Wiesbaden, Germany; University Hospital, Rostock, Germany; Rot-Kreuz-Krankenhaus, München, Germany; University Hospital, Kiel, Germany; Henrietten-Stift, Hannover, Germany; Krankenhaus Berg, Stuttgart, Germany; University Hospital, Münster, Germany; Markus-Krankenhaus, Frankfurt, Germany; Bethesda-Krankenhaus, Essen, Germany

Introduction: P with an early partial (PR) or complete (CR) clinical remission after 2 cycles of PCT have a 16fold higher chance to obtain a pathological CR at surgery compared to patients with no rapid tumor response (von Minckwitz et al. SABCC 2000). This in-vivo chemosensitivity test was integrated into the trial design of the GEPARTRIO-study.

Methods: P with primary operable (T2-3, N0-2, M0) or locally advanced (T4 a-d, N0-2, M0) breast cancer are being treated with 2 cycles of TAC (Docetaxel 75mg/m^2, Adriamycin 50 mg/m^2, Cyclophosphamid 500 mg/m^2 day 1, q day 22). In case of reduction of the palpable breast tumor area of > 49%, further 4 cycles of TAC were administered. If tumor reduction was less, P were randomized to either further 4 cycles of TAC or 4 cycles NX (Vinorelbine 25 mg/m^2 day 1 + 8, Capecitabine 2500 mg/m^2 day 1-14, q day 22).

Results: Since October 2001 204 patients were recruited into the trial by 20 institutions. 183 patients had operable and 21 an inoperable breast disease. To date 124 patients were evaluable after the 2nd cycle and 87 (70.2%) had an early remission of the tumor. Clinical response correlated strongly with ultrasound evaluation. 37 non-sufficiently responding patients were randomized to either TAC or NX. So far no drug related serious adverse event (SAE) during NX was reported. During TAC 7 events of grade 3/4 neutropenia and 6 events of febrile neutropenia are documented. Further SAE during TAC were dyspnoea (1), pulmonary embolism (1), thrombopenia (1). No treatment discontinuations are reported so far.

Conclusions: Only 30% of Patients do not respond sufficiently after 2 cycles of TAC. This may represent an unfavorable group of patients where the change to a non-cross resistant regimen has to be tested. NX appears to be a feasible salvage treatment. Results on pCR-rates will be presented.

157 Oestrogen receptor directed primary systemic therapy: a randomised trial compared with conventional therapy in operable breast cancer.

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Between January 1990 and October 1995, 171 women with large operable (> 3cm, T2, T3, N0, N1, M0) breast cancer were randomised between

• (PST) primary systemic therapy for 3 months directed by ER (n=85):
- ER ≥ 20fmol/mg cytosol protein: hormonal therapy (goserelin 3.6mg/month, or tamoxifen 20mg/day as per menopausal status).
- ER <20: prednisolone 40mg po x 5 days, cyclophosphamide lg/m² iv, adriamycin 50mg/m² iv every three weeks x 4 cycles.
- Patients progressing on hormonal therapy switched to chemotherapy.

• (CT) Conventional treatment; surgery +/- radiotherapy followed by late 1980's standard adjuvant chemotherapy (n=86):
- 6 cycles of CMF chemotherapy for premenopausal node +ve patients
- tamoxifen 20mg a day for 5 years for all other patients.

40 PST and 47 CT patients have relapsed at a median follow-up of 8 years:

Relapses	Chemo.	Endo	Endo→chemo	6 yr DFS	6 yr OS
Clinical CR	5/10	0/0	0/1	57%	57%
Path CR	2/5	0/0	0/0	80%	80%
Clinical PR	5/16	4/19	1/5	79%	77%
SD/ PD	5/8	13/25	4/4	41%	48%
PST Node -ve	6/18	3/18	0/2	83%	85%
PST Node +ve/UK	9/11	21/33	5/8	50%	50%
CT Node -ve		15/37		64%	70%
CT Node +ve/UK		32/49		42%	45%

On an intent to treat analysis there was no significant difference in outcome, after 4 years the curves diverge in favour of PST (p<0.1).

This study with 8 years of follow-up shows no disadvantage to using PST directed on the basis of ER status and suggests that pathological response in the nodes matters more than the response at the site of the primary tumour.

158 Pathological complete response is not the best prognostic factor in patients with breast cancer treated by preoperative chemotherapy.

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Aim of the study

Preoperative chemotherapy (pCHT) changes established prognostic markers. This study evaluated the prognostic value of pathological complete response (pCR) in comparison to other prognostic factors (PF) after a follow up of 6 years.

Material and methods

156 patients with locally advanced breast cancer (clinically T2 or T3-tumors, M0) obtained pCHT with Epirubicin/Cyclophosphamid (3-4 cycles, 90/ 600mg/m2 every 3 weeks)

Results

77% of patients showed a remission of greater than 50% (n=120) and a pCR in 6.4% (n=10). Breast conserving surgery was performed in 68% (n=106) of patients. Metastasis was seen in 38.4% (n=60) of patients but only in 2 of patients with pCR (p=0.146).

Significant prognostic factors for DFS (disease free survival) were the clinically assessed tumor size before pCHT (p=0.009), the clinical nodal status before pCHT, (p=0.041), grading (p=0.005), the histological tumor size after pCHT (p=0.001), the histological axillary lymph node status after pCHT (p=0.001) and the clinical response of the tumor to pCHT (p=0.032). Regarding OAS (overall survival) only the grading and the pathological lymph node status proved to be statistically significant. Occurrence of pathological complete response did not prove to be a statistically significant marker.

Regarding DFS grading (p<0.001, RR=2.49), histological tumor size after pCHT (p<0.001, RR=2.49) and the histological lymph node status (p<0.001, RR=2.11) proved to be independent prognostic factors in multivariate regression analysis. Looking at OAS histological lymph node status (p=0.008, RR=1.79) as well as grading (p=0.001, RR=3.48) remained independent prognostic factors.

Summary

The most important prognostic factors after 6 years of follow up are the pathological lymph node status and the grading. Occurrence of pathological complete response was no statistically significant prognostic factor. Kind of pCHT and number of cycles could be responsible for this finding.

159 Breast conserving surgery after neoadjuvant chemotherapy paclitaxel + doxorubicln vs fluorouracil + doxorubicin + cyclophosphamide in locally advanced breast cancer.

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Introduction: Primary chemotherapy is increasingly used to treat large breast cancer with the aim of downstaging of primary tumor. Methods: One hundred and three patients (pts) with locally advanced breast cancer (T2N2, T3NI, T4NO-I) received 4 cycles of neoadjuvant chemotherapy-Paclitaxel 200 mg/ m2 + Doxorubicin 60 mg/m2 (PD-regimen), every 3 weeks (51 pts) vs 4 cycles of neoadjuvant S-Fluorouracil 600 mg/m2 + Doxorubicin 60 mg/m2 + Cyclophosphamide 600 mg/m2 (FAC), every 3 weeks (52 pts). Tumor response to preoperative chemotherapy was assessed after 4 cycles by palpation and mammography. Then appropriate surgery was performed. Surgical specimens were examined for the presence of microscopic residual tumor.

Results: One hundred and three patients were included (SI - PD group, 52 - FAC). Clinical tumor response:

Preoperative treatment	Complete response	Partial response	Stabilization	Progression
PD	33.3%	50.9%	15.7%	0
FAC	11.5%	61.5%	26.9%	0

Pathological complete response (pCP.) was observed in 13 pts (25.4%). received PD, and 5 pts (9.6%), received FAC (P=0.003). The variables associated with pCR were negative ER status and clinical complete response. Breast conserving surgery was realized in 18 pts (35.2%) in PD group and 15 pts (28.8%) In FAC group (P=0.65). At a median follow-up of 24 months local recurrence (LR) after conserving surgery was seen in 1 patient (5.5%.) of PD group and 4 (26.6%) of FAC group (p=0.08). No recurrence were seen in patients with a complete pathological response. Conclusion: Extent of clinical and pathological response to neoadjuvant chemotherapy may be useful in identifying patients at risk of developing LR.

160 Quality of life during primary chemotherapy for breast cancer with continuing cyclophosphamide, vincristine, adriamycin and prednisolone versus sequential docetaxel: a randomised trial.

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Background:We have recently demonstrated enhanced clinical and pathological responses in women who received 4 cycles of anthracycline-based chemotherapy followed by 4 cycles of docetaxel compared with women given 8 cycles of the anthracycline regimen. The relative effects of these neoadjuvant regimens on various aspects of quality of life have not been documented previously. The aim of this study was to compare the effects of the two regimens on key parameters of quality of life.

Methods: 131 patients with large or locally advanced breast carcinoma gave written, informed consent to participate in the quality of life study. They all received 4 cycles of cyclophosphamide, vincristine, adriamycin and prednisolone (CVAP). The 92 women with complete or partial response (UICC criteria) were randomised either to 4 further cycles of CVAP, or to 4 cycles of docetaxel. These randomised patients were the subjects of the quality of life analysis. Before each cycle of chemotherapy, and 3 weeks after the 8th, they completed standardised measures of various components of quality of life, including the Hospital Anxiety and Depression Scale (HADS), the Rotterdam Symptom Checklist (RSCL) the Profile of Mood States (POMS) and a measure of various side-effects. In addition, they completed the Functional Assessment of Cancer Therapy (Breast Cancer version) (FACT-B) before cycles 1 and 5, and 3 weeks after cycle 8. The data for women who had been randomised were analysed using repeated measures analysis of covariance (with pre-cycle 5 as the covariate) or, in the case of FACT B, simple analysis of covariance.

Results: Even before adjusting for multiple comparisons, the groups did not differ significantly on any of the standardised measures, or their subscales. The only differences were in the following side-effects: patients receiving sequential docetaxel had less nausea (F=5.48, p=0.009) and more nail problems (F=10.114, p=0.002).

Conclusions: This study demonstrates that the effects on quality of life of sequential CVAP and docetaxel are very similar, and that the enhanced clinical and pathological responses to sequential docetaxel are not at the expense of key parameters of quality of life.

161 Analysis of prognostic impact of time interval between the end of neoadjuvant chemotherapy (CT) and initiation of local/regional treatment in operable breast cancer.

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The aim of this study was to assess if delay of the local control may have an adverse effect on local relapse rate and survival in a retrospective analysis of our database.

Patients and Methods: Between 1981 and 1994, 1006 patients (pts) had received neoadjuvant CT for operable T2 or T3 < 7 cm, N0 or N1, M0 tumors. Median follow-up was 130 months (14-240). Time interval (TI) was determined between last day of CT delivery and day of surgery or start of radiotherapy (RT). Median age was 47 yrs (24-71), 76% were premenopausal, 67% of pts had T2 tumor, 53% N0, 59% ER+, 56% PR+, 21% SBR 3. Neoadjuvant CT contained anthracycline in 87% of cases. Median number of cycles was 4. Local/regional treatment, according to clinical response, consisted in surgery +/- RT in 471 pts (47%), RT alone in 331 pts (33%) and RT followed by surgery in 204 pts (20%). Histologic response was available in 321 patients. TI before local treatment was < 21 days (d) in 18% of pts (group 1), between 21 and 42 d in 60% (group 2) and > 42 d in 22% (group 3). Patients treated before 21 d had significantly higher tumor size, tumor grade and clinical nodal involvement.

Results: Ten-year overall survival in groups 1, 2 and 3 were respectively 63%, 68% and 62% (p=0.17). There was no influence of the TI on local relapse-free survival. Complete pathological response rate was 11% in group 1 versus 6.5% in groups 2 and 3 (p=0.44). In a multivariate analysis, prognostic factors were for survival : clinical nodal status, menopausal status, age < 40 yrs, clinical response to CT, tumor grade and ER - and for local relapse: age < 40 yrs, clinical nodal status, tumor size, estrogen receptor status and lack of surgery. Paradoxically, in subgroup analysis for survival, TI > 42 d had a significant adverse effect only in premenopausal pts with ER + tumors.

Conclusion: In this retrospective analysis, we did not observed any significant adverse effect of a long TI before local/regional treatment due to a tumor regrowth. However, the low number of pts in subgroups with higher TI (> 42 d) and aggressive tumors does not allow to draw definitive conclusions.

162 Neoadjuvant treatment of women with breast cancer utilizing docetaxel and vinorelbine with growth factor support.

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We report preliminary results of two trials. In the first study women with stage IIA-IIIB and locoregional stage IV (LCRSIV) breast cancer irrespective of HER2 status were treated with docetaxel (D) 60 mg/m^2 and vinorelbine (V) 45 mg/m^2 administered every two weeks with G-CSF and quinolone prophylaxis for six cycles prior to surgery (TNAC). In the second trial women with stage IIB-IIIB and LCRSIV breast cancer and HER2 overexpression (by fluorescence in situ hybridization) were treated with the same regime plus concurrent weekly trastuzumab (TNAC/herceptin) for twelve weeks followed by surgery. Twenty-seven patients (pts) have been enrolled (TNAC-21, TNAC/Herceptin-6). Age range 27-66. Stage II-15, stage III-11, LCRSIV-1. Over 140 cycles have been administered. Relative delivered dose intensity (RDI) for D 95% and RDI for V 94%. Grade ¾ or severe toxicity has included neutropenic fever 8/27 (30%), epiphora 4/27 (15%) and nail changes 3/27 (11%). Early on constipation was seen in two pts prompting incorporation of prophylactic laxative treatment and this toxicity has not been significant since. Other toxicities have been minimal with this regimen being well tolerated by all patients. 26 pts are evaluable for clinical response: there were 12 (46%) complete responses (cCr) and 13 (50%) partial responses (PR), one pt has progressed in the breast. Pathological data is available on 20 pts: 7 pts (35%) had a pathologic complete response (pCR) by NSABP criteria, 2 additional pts had less then 5 mm of residual cancer. Our preliminary data suggest that this dose intense neoadjuvant regime was well tolerated and can be administered safely. Dose intensity is maintained in the majority of patients. The current overall clinical response rate is 96% with a cCR rate of 46%. The pCR rate of 35% with 45% of patients having less then 5 mm of residual tumor after neoadjuvant treatment is encouraging. Active enrollment continues at all centers.

163 High response rate with Navelbine (NVB) and tamoxifen (TAM) as neo-adjuvant treatment for locally advanced breast cancer (LABC) in elderly patients.

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Background : In elderly pts some comorbidities can impact negatively on treatment decision. NVB as single agent is among the most active drugs in breast cancer and shows synergism with TAM "in vitro". Based on this favorable data, in March 2000 we implemented an international open phase II study in 11 countries.

The objective was to combine both drugs in order to have at least similar results as with anthracyclines with minimal side effects. We present an intermediate analysis on the first 50 pts.

Methods : Pts with confirmed breast cancer, 60 years and ER (+) were eligible. They received NVB 30 mg/m2 D 1 & 8 q 3 weeks (max 6 cycles) concomitantly with TAM, 20 mg daily. Then patients underwent surgery plus local RT +/- CT.

Results : Median age was of 68 years (range 60-94). Pts presented large tumors at study entry with a median diameter of 6.2 cm. All pts were ER (+) but 22% had PgR (+).

Among the 50 pts analyzed, only 49 were evaluable for safety (1 patient too early). A total of 247 cycles were administered. The median DI of NVB was 17,8 mg/m2/w (median RDI: 89%). Only 54 (27%) cycles of NVB were delayed (50% of the pts). The commonest hematological toxicity was Neutropenia (grade 3-4) in 17% of the pts, complicated with fever and infection in only 1 pt (who died). Non-hematological toxicities included severe tumor pain treated by narcotics (4%), grade 3-4 constipation (4%), infection (2%), peripheral neuropathy (2%) and leg phlebitis (2%). 44 out of 50 pts are evaluable for efficacy (2 pts were withdrawn for toxicity after 1 and 2 cycles; one died before tumor evaluation and for 3 are too early). OR in the evaluable pts was of 84% (CI 95% [73.2-94.8]) (4 pts CR, 33 pts PR -not confirmed in 7 pts-and 6 pts NC). Only one progression was noticed. Surgery was performed in 87% of them (BCS in 17%) with a median delay between the end of neo-adjuvant and surgery of one month (range 0,5-4,6). The histological examination showed a pCR in 9% of pts.

Conclusion : The combination of NVB and TAM as neo-adjuvant treatment is an effective and well tolerated treatment, with high rate of clinical response in patients with bulky disease. Moreover, this treatment allowed an optimal multidisciplinary approach without delaying timing of surgery.

164 Neoadjuvant docetaxel (Taxotere®, DOC) followed by epirubicin (Ellence®, EPI) in stage IIB, IIIA,B breast cancer: mitogen-activated protein kinase (MAPK) and c-Jun-N-terminal (JNK) stress-activated protein kinase (SAPK) pathways are activated after DOC treatment.

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Rationale: Neoadjuvant chemotherapy provides a direct assessment of tumor response and sequential biopsies for molecular correlative studies. Taxane-mediated activation of p44/42; p38 MAPKs and JNK/SAPK pathways is observed in breast cancer model systems, and is associated with the induction of apoptosis in some studies. Methods: From Oct 2001 to May 2002, 16 patients (pts) with stages IIB (n=6), IIIA (n= 4), IIIB (n= 5), and IV with intact primary tumor (n=1) received DOC 100 mg/m2 every 21 days for 3 cycles (C1-3) followed by EPI 100 mg/m2 every 21 days for 3 cycles (C4-6). Core biopsies were obtained pre and post 3 cycles of DOC. Signaling through p44/42; p38 MAPKs and JNK/SAKP pathways were evaluated in a blinded fashion in pre and post-DOC biopsies using immunofluoresence with phospho-specific and control antibodies. Results: Pt characteristics: median age 53 years (range 42-66); postmenopausal (63%); estrogen receptor negative (56%); and HER2-neu 3+ (50%). Thus far, 13 (81%) pts have completed C1-3 of DOC, and 9 (56%) have completed C4-6 of EPI. Grade 3,4 toxicities all during C1-3 included: 7 (44%) with febrile neutropenia; 1 (6%) grade 3 diarrhea; and 1(6%) grade 3 rash. Only 1 (6%) pt has progressed on neoadjuvant chemotherapy. Response as assessed by definitive surgery in 8 (50%) pts shows: 2 (25%) pathological complete response (pCR) in the breast and axillary nodes; 4 (50 %) pCR in breast with nodal positivity; and 2 (25%) pCR in nodes but invasive tumor present in the breast. In 5 pairs of pre and post DOC biopsies qualitative increases in phospho- p44/42, phospho-p38, and phospho-JNK/SAPK after DOC were observed, indicating activation of these pathways. Conclusions: Preliminary results of neoadjuvant DOC followed by EPI suggest the regimen is active and tolerable. To our knowledge this is the first demonstration of activation of MAPK and JNK/SAPK pathways in response to DOC in human breast cancer. The trial will continue until a target accrual of 25 pts and the extent to which MAPK and JNK/SAPK activation correlates with pCR will be presented.

165 Weekly paclitaxel as a neoadjuvant setting for patients with breast cancer.

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Background: Paclitaxel is known as one of the most active agents for the treatment of breast cancer, but the effectiveness of weekly method as a neoadjuvant setting is not shown.

Patients and Methods: Patients with primary operable breast cancer (>3 cm of invasive tumor) were eligible for the current phase II study. Weekly paclitaxel therapy consisted of 12 courses of paclitaxel 80 mg/m^2 every week, followed by radical surgery. Before treatment, cancer tissues were obtained under written informed consent using core needle biopsy. Samples were utilized for pathological examinations and cDNA microarrays following laser-capture microdissection of tumor tissues and normal tissue. From March until November 2001, 42 patients have been entered into the study. Twenty-three patients (median age 50 y, range 26-67 y) have finished treatment and have been evaluated. Eleven patients displayed stage II, 9 patients displayed stage IIIA; and 3 patients displayed stage IV (protocol violations).

Results: The regimen was well tolerated by the majority of patients, and a median of 11.0 courses (range 3-12) was received. Grade III/IV toxicities included granulocytopenia (9%), liver dysfunction (4%) and neuromotor toxicity (13%). Overall clinical response rate was 70% (16/23), with 9% (2/23) complete responders, and two pathological complete remission (9%) was observed. Eight of the 16 responders underwent breast-preserving operations. Correlations between responses and gene expression patterns are under investigation.

Conclusions: Weekly Paclitaxel (80 mg/m^2/q1wk X 12) seems to offer a highly active and safe regimen for patients with relatively large invasive tumors as a neoadjuvant setting.

Discussion: Since the effectiveness is not consistent for individual cancers, our intention of the present study was to clarify the correlations between responses and gene expression patterns using cDNA microarrays. The results by cDNA microarrays will be available at presentation.

166 Neo-adjuvant chemotherapy with weekly docetaxel (taxotere) in poor prognosis locally-advanced breast cancer (LABC).

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Introduction: Locally advanced breast cancer is a public health issue in Brazil. Phase II studies of docetaxel on a weekly schedule in metastatic breast cancer have shown encouraging results and ongoing studies are evaluating this regimen in the adjuvant setting. We report a multicentric Brazilian study of neoadjuvant chemotherapy with weekly docetaxel in unfavourable LABC. Patients and Methods: Patients (pts) with stage IIIA(T3) or IIIB breast cancer were treated with docetaxel 36mg/m2 weekly for 6 weeks followed by 2 weeks rest for 2 cycles, followed by breast surgery. Objectives: (1) Overall response rate (ORR); (2) pathologic response; (3) safety profile; (4) correlation of HER-2 status with response. Results: Thirty-seven pts were enrolled between March and September 2000. Median baseline tumor size: 68.5 mm (30-150mm); clinical stage: 35% IIIA, 65% IIIB; ER status: 60% ER+, 35% ER-, 5% ER unknown; HER-2 status: 16% HER-2 ++ and 19% HER-2 +++ (DAKO). All pts were evaluable for safety. Of a total of 399 infusions, grade III neutropenia (8%) was reported as the only serious hematologic toxicity. The main nonhematologic toxicity was grade III diarrhea (11%). Response data was available for 36 pts (exclusion due to protocol deviation). The ORR was 67 % (95% CI: 51-82%) with a PR of 47% and a CR of 19%. Pathologic CR occurred in 2 patients (5%). There was no significant difference in response associated with HER-2 status. Conclusion: Weekly docetaxel is effective and well-tolerated in the treatment of poor-prognosis patients with LABC. This regimen resulted in a clinical response of 67% and pathologic CR of 5%. Our ongoing study is evaluating the combination of weekly docetaxel and trastuzumab (Herceptin) in patients with LABC that overexpress HER-2.

Supported by Aventis Pharma Brazil.

167 Prospective pilot study of the preoperative use of celecoxib (Celebrex™) and FEC for the treatment of locally advanced breast cancer.

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Background: Neoadjuvant treatment of breast cancer with FEC is well tolerated among Asian patients and it allows more breast-conserving surgery to be performed. COX-2 is over expressed in breast cancer and it is also a novel target of treatment. Celecoxib, a COX-2 inhibitor, has anti-angiogenic and apoptotic effects.

Objective: To study the clinical and pathologic responses, changes in the angiogenesis markers and tolerability to FEC + celecoxib or FEC alone.

Materials and Methods: 3 cycles of FEC (5 FU 500mg/m2, Epirubicin 75mg/m2, Cyclophosphamide 500mg/m2) with or without celecoxib (400mg b.d.) were given every 3 weeks to women with histologically proven LABC. End point assessments include clinical and pathologic responses, changes in tissue and serum angiogenesis markers and tolerability.

Results: From June 2001 to November 2001, a total of 32 patients were recruited. Sixteen patients in each arm received either a combination of FEC and celecoxib or FEC alone. The mean age was 45.6 (SE, 2.7) years. Clinical response rate of 62.5 % (cCR 6.3%,cPR 56.3%), was achieved in the FEC alone arm and 81.3% (cCR 18.8%, cPR 62.5%) in the combined arm of FEC and celecoxib. The pathologic response rate was 87.5% (pCR 12.5%, pPR 75%) in the combined arm and 62.5% (pCR 6.3%, pPR 56.3%) in the control arm. In the combined arm, the mean tumor size decreased from 4.52 (SE,0.32) to 2.04 (SE, 0.31) cm 31.3% of the patients chose to undergo conservative (lumpectomy) surgery and the rest opted for mastectomy.

Data on COX expression, and angiogenesis-hypoxia drug resistance markers are being analyzed.

The regimens were well tolerated. No clinical cardiac toxicity was detected in both arms.

Discussion: The combination treatment of celecoxib and FEC is effective and potentially allows more breast-conserving surgery to be performed in patients with LABC. This is an ongoing study and will be expanded to include more patients. Angiogenesis marker test results will be presented.

168 A naturally occurring point mutation alters ERα crosstalk with regulatory signaling pathways.

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Estrogen receptor α (ERα) belongs to the superfamily of ligand-dependent transcription factors. It is well accepted that in addition to being activated by ligand binding, ERα activity is also regulated by diversified signaling pathways, such as that of Histone Acetyl Transferase and G-proteins. Recently we have isolated a somatic ERα mutation in human breast hyperplasias and invasive breast cancers. This mutation (Lys 303 to Arg, K303R) disrupts the major acetylation site of ERα at K303. Since we have shown that this mutation alters ERα interactions with the p160 family of ER coactivators, we hypothesized that it might also alter ERα crosstalk with other signaling effectors. We found that wildtype ERα activity could be inhibited by a component of the nucleosome remodeling and histone deacetylase complexes, called metastasis-associated protein 1-like 1 (MTA1-L1) using transient transactivation assays. Furthermore, we found that the activity of the K303R ERα mutant is refractory to MTA1-L1 inhibition. We also determined that MTA1-L1 could enhance deacetylation of wildtype ERα using in vitro deacetylation assays. Finally we demonstrated that a selective HDAC inhibitor, sodium butyrate could restore MTA1-L1 repressed wildtype ER activity. Taken together, our results suggest that the K303R mutation alters the crosstalk between ERα and diversified ERα pathways that normally downregulate ER signaling. We hypothesize that this loss of regulation may provide additional selective forces for ER-mediated tumor progression.

169 Evidence for transcription factor cross-talk in breast cancer: identification of estrogen-induced AP-1-dependent genes.

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Estrogen has been documented to play a critical role in the etiology and progression of breast cancer. However, the mechanisms by which estrogen stimulates breast cancer growth are not completely understood. The estrogen receptor (ER) mediates most the effect of estrogen by activating classical pathways through direct interaction with DNA at an ERE or alternatively ER can activate non-classical pathways through interaction with other transcription factors such as AP-1 or SP-1. We have shown that Tam67, a cJun dominant-negative construct lacking the cJun transactivation domain, blocks IGF-, EGF-, heregulin- and estrogen-induced growth of breast cancer cells. To investigate estrogen's ability to stimulate growth through cJun and its dimerization partners, we measured estrogen's ability to stimulate gene expression in the presence of AP-1 blockade. The goal of this study is to identify the estrogen-induced growth-regulatory pathways that are dependent on AP-1. Our results show that AP-1 blockade does not affect estrogen-induced activation of an ERE reporter construct or the expression of the ERE-dependent gene PS-2. However AP-1-blockade does affect several other known estrogen-induced genes (PR, E2IG1 and Cyclin D). We also observed that estrogen stimulates the transcription of several AP-1 dependent genes (MMP1 and Fra-1). We have also queried microarrays to identify additional novel estrogen-regulated and AP-1-modulated genes in breast cancer cells. A proportion of these estrogen-regulated genes were also modulated by AP-1 blockade. These genes appear to be both estrogen-regulated and AP-1-dependent. Such genes are candidates for regulation through non-classical pathways of estrogen induction. We are currently investigating the kinetics of these estrogen-modulated genes, their promoters and their involvement in regulating estrogen-induced growth. These studies suggest that many estrogen-regulated genes are dependent on transcription factor crosstalk between ER and other transcription factors, such as AP-1. A better understanding of how estrogen stimulates breast cell growth will provide the foundation to develop more effective agents for treatment and prevention of breast cancer.

170 A link between the ER negative status of human breast cancers and accelerated ER proteolysis via cross talk with Her2 and cSrc.

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Background: About 1/3 of breast cancers are estrogen receptor (ER) negative and this confers a poor prognosis. Estrogen-ER binding leads to transcription of target genes. In addition, estrogen-ER binding activates signaling pathways that modulate ER function through ER phosphorylation. Estradiol binding also activates ubiquitin dependent ER proteolysis. We investigated the cause underlying ER loss and the aggressive behavior of ER negative breast cancer.

Methods and Results: Estradiol stimulation of ER positive MCF-7 cells dramatically reduced the ER half-life (t1/2) within one hour. Proteasome inhibition prevented the estradiol-stimulated loss of ER protein. Repletion of serum and estradiol together caused a more rapid loss of ER protein, suggesting a synergistic effect of estradiol and growth factor signaling on ER proteolysis. Src inhibition increased ER protein levels and transfection of activated Src or of Her2 into MCF-7 shortened the ER t1/2. Transfection of Her2 together with Src further lowered ER levels. Src action on the ER may both condition it for interaction with mediators of its own proteolysis (the ubiquitin conjugating enzyme and the E3 ligase) and activate ER transcriptional function. Effects of Src transfection on ER transcriptional activity are being assayed. Neither MAPK nor PKB appear to activate ER proteolysis. The "ER negative" BT-20 and weakly ER positive MDA-MB361 cell lines show marked activation of cSrc kinase compared to MCF-7. When BT20 and MDA-MB361 lines were serum and estrogen starved or treated with proteasome or Src inhibitors, weakly detectable ER protein was significantly increased. The ER t1/2 in both BT20 and MDA-MB361 lines is shorter than that in MCF-7. We have identified a putative Src-phosphorylation site on the ER whose mutation prevents estrogen dependent ER-ubiquitination and degradation. To confirm the link between Src and ER negativity, ER mRNA and protein and Src activity will be assayed in human breast cancer tissues.

Discussion: Our data suggest a model whereby estrogen-ER binding causes cross talk with Src which phosphorylates the ER and activates its proteolysis. Oncogenic EGF or Her2 signaling in breast cancers would act synergistically with cSrc to phosphorylate the ER, leading to constitutive ER proteolysis. Thus, the very mechanisms that mediate ER negativity also mediate increased mitogenic activity in this aggressive form of breast cancer.

171 SAFB1 inhibits estrogen receptor activity by recruiting the Ret finger protein: a novel mechanism of estrogen receptor corepression.

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We have previously shown that the nuclear matrix protein SAFB1 binds to the estrogen receptor (ER) and functions as an ER corepressor (Oesterreich et al. Mol Endo 2000). Our finding of a high loss of heterozygosity at the SAFB locus and our demonstration of the growth inhibitory effects of SAFB in tissue culture cells suggests that SAFB functions as a tumor suppressor. We believe that the tumor suppressor activity of SAFB is partly due to its function as an ER corepressor. We had hypothesized that SAFB1 represses ER activity by recruiting chromosome remodeling protein complexes to ER target promoters, possibly in addition to its effect on RNA processing-SAFB1 has an RNA binding domain (RRM), and we have shown that this domain can indeed bind RNA. Furthermore, in recent years a number of steroid receptor cofactors have been identified that can bind RNA (hnRNPU, p68, p72, TLS, Sharp, PSF, NonO/p54^{nrb}), or are themselves RNA molecules (such as SRA). However, a SAFB1Δ RRM mutant can still repress ER activity in transient transfection assays. Additionally, cotransfection of SAFB interacting RNA processing factors such as Tra2 and AUF1, did not alter the levels of SAFB1-mediated repression. Thus, our assays suggest that RNA processing might not be the major mechanism of repression by SAFB1. Next we employed transient transfection assays using a Gal4 DBD responsive luciferase reporter construct. Using a full length SAFB1 Gal4 DBD fusion protein, we observed a strong repression (7-fold) of the reporter activity and demonstrated that SAFB1 has two distinct transcription Repression Domains (RD1 and RD2). A yeast two-hybrid screen to identify repression domain interacting proteins identified the Ret Finger Protein (RFP) as one of these interacting proteins. RFP is a known transcriptional repressor that interacts with the polycomb group gene Enhancer of Polycomb. Using the yeast two-hybrid assay, we demonstrated that the coiled-coil repression domain in RFP interacts with the SAFB1 RD1. Given that polycomb group genes are involved in gene silencing rather than short-term transcriptional regulation, we suggest that SAFB1 may repress ER activity via a mechanism not previously connected with nuclear hormone receptor action.

172 Molecular classification of endocrine disrupters based on the shape of the estrogen receptor (ER)α complex.

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Background: The recent molecular classification of estrogens [Jordan et al., Cancer Research 2001:61;6619-23] raises the possibility that different shaped endocrine disrupters (EDs) have different mechanisms of action. The shape of the ER complex can be predicted by the activation of the transforming growth factor (TGF)α gene by comparing wild type (wt) ER and D351G ER action. We have used environmental estrogens to determine whether they are equally estrogenic or classifiable based on their mechanisms of action.

Methods: Xenoestrogens [coumestrol, genistein, bisphenol A, methoxychlor and its mono (Met1) and didemethylated metabolites (Met2)] were evaluated in the TGFα assay as described above. Each compound was tested in MCF-7 cell growth assay to confirm estrogen-like activity.

Results: The fifty percent effective concentration (EC50) on the MCF-7 growth assay was as follows: estradiol (3x10-12M), coumestrol (3x10-10M), genistein (3x10-9M), bisphenol A (3x10-7M) and Met2 3x10-9M). Response on the TGFα assay depended on the shape of the ligand. Planar (class I) compounds such as coumesterol or genistein activated TGFα gene expression in the wt ERα. Nonplanar (class II) compounds such as bisphenol A and Met2 also stimulated expression in the wild type ER. However, only planar compounds stimulated gene expression in the D351G ER cells. This was in contrast to the nonplanar compounds, bisphenol A and Met2 of methoxychlor, which did not activate gene expression.

Discussion: EDs have different activities at target sites depending on the shape of the ER complexes. This finding suggests that the ED:ER complex recruits different coactivators and corepressors . We demonstrate that methoxychlor is a proestrogen that requires demethylation to produce estrogen action. As each of the methyl groups are removed, these derivatives become more estrogenic with the wt ER. However, these derivates are sufficiently nonplanar to displace helix 12 and block activating function 2 (AF2) to create a dependence on the AF-2b site on the ER that synergizes with AF-1. The AF-2b site requires D351 to permit estrogen-like gene transcription. When this site is altered as with the D351G ER, the estrogenic function is lost. These data extend the molecular classification of estrogens based on three dimensional shape.

173 The shape of the estrogen receptor (ER) beta-ligand complex predicts the agonist and antagonist actions of endocrine disrupters in target tissues.

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Background: Endocrine disrupters (EDs) can interact with the ER in hormone responsive tissues. We have demonstrated that the shape of the ligand-receptor complex may play a role in determining the molecular response at target sites. (Jordan et al, Cancer Research 61, 2001 pp.6619-6623) The behavior of the ER-ligand complex can be predicted based on the activation of the TGFα gene in cells transfected with wild type ER and ERβ. It is possible that the proportion of the various isoforms (α and β) of the estrogen receptor (ER) may be responsible for the observed response to these substances. We have used a series of environmental estrogens to predict their response in ERβ dominant tissues. Furthermore, we classify these compounds based on their molecular mechanism of action.

Methods: Xenoestrogens, coumestrol, genistein, bisphenol A, methoxychlor and its didemethylated derivative (MethOH) were evaluated in the TGFα assay as described above with the modification that MDA-MB-231 breast cancer cells were stably transfected with cDNA for ERβ. Compounds were tested in T47D:A18 cell growth assays to confirm their estrogen-like activity and relative potencies.

Results: Responses in the TGFα assay separated compounds into two groups based on their structure. Planar (class I) compounds like estradiol, coumestrol, and genistein activated TGFα gene upregulation in the ERβ transfectants. Nonplanar (class II) compounds such as bisphenol A, methoxychlor and its (mono and di) demethylated derivatives did not upregulate the TGFα gene. The activity of these compounds on the T47D growth assay were consistently estrogenic.

Discussion: Full activation of the ER in target tissues depends on the synergy of activating functions (AF1 and AF2) in the ligand receptor complex. ERβ lacks a functional AF1 domain and relies on the AF2 function to maintain its transcriptional activity. Compounds (class II) that will inactivate the AF-2 function by displacing the helix 12 region of the ER will produce an antagonist complex. EDs that are three dimensional and silence AF2 would be predicted to act as estrogen antagonists at sites exclusively positive for ERβ. In contrast, class I estrogens are planar and act as traditional estrogens at ERβ sites.

174 A novel functional estrogen receptor reporter gene assay (FERA) demonstrates functional differences in oestrogen receptor activity in primary and metastatic breast cancer cells.

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Background: Expression of estrogen receptor (ER) is used to indicate response to endocrine therapy. However, assays e.g. immunohistochemistry (IHC), that meausre ER and ER regulated progesterone receptor (PR), predict response in only 40-60% of cases. We have developed a functional assay (FERA) based on the interaction of ER with estrogen response elements (EREs). The assay uses an adenovirus containing 2 EREs linked to a lac-Z reporter (ERE-lacZ).

Methods: Breast cancer cells were purified from surgical specimens and ascites obtained from patients. The cells were infected with the ERE-lacZ adenovirus and cultured in medium containing 10nM estrogen (E2), 100nM tamoxifen (OHT), or 100nM ICI 182,780 (ICI, a complete ER antagonist). A colorimetric assay was used to assess reporter activity. The results of FERA were compared with IHC.

Results: In purified cells from patients with primary breast cancer, oestrogen stimulation was seen in 8/12 ERα positive samples whereas activation was observed in only 1/6 ERα negative preparations. Only 2/5 ER+/PR- samples showed stimulation of LacZ activity. In cells from ascites from patients relapsing after endocrine therapy, we observed 6 of 10 ERα positive samples showing activity and 2 cases showed stimulation of reporter activity by tamoxifen.

Conclusions: This is a reproducible, sensitive, functional assay for ER. It shows that some patients who have become resistant to several anti-endocrine agents still have functional ER.

175 Relationships of the prostaglandin E (PGE) content in primary breast cancer tumors with estrogen and progesterone receptor levels.

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Prostaglandins play an important role in regulation of aromatase activity, but relationships between PGE synthesis and steroid hormone receptors levels, which are targets of locally formed hormones, in breast cancer were not study enough. Methods: PGE levels were measured in primary tumors of the 134 previously not treated patients with breast cancer (stage I, II - 58 patients, stage III - 76 patients), in 58 breast tissue specimens without evidence of malignancy (normal tissue) and in 12 benign tumors of the breast. Simultaneously in primary cancer tumors were studied levels of estrogen receptors (n=124) and progesterone receptors (n=118). PGE content in samples was measured with using of Kits for radioimmunoassay of PGE after preliminary extraction by ethylacetate. Steroid hormone receptors levels were studied by specific binding of labeled hormones with receptors. Results: Mean levels of PGE in primary breast cancer tumors are significantly elevated. There was back relationship between PGE and estrogen receptors (ER) contents. The PGE content in ER-negative (ER<10 fmol/mg of protein) tumors (n=65) was 68,7 ng/g of tissue, in tumors with moderate levels of ER (10-50 fmol/mg of protein) (n=43) - 52,0 ng/g of tissue, and in tumors with high levels of ER (ER > 50 fmol/mg of protein) (n=16) PGE content was 35,7 ng/g of tissue ($p < 0,05$). On the other hand, PGE content in PR-negative tumors (PR<10 fmol/mf of protein) (n = 60) and in tumors with moderate levels of PR (10-50 fmol/mg of protein) (n = 38) was significantly ($p < 0,05$) lower than in breast cancer tumors with high content of PR (PR > 50 fmol/mg of protein) (n = 20): 57,1; 53,6 and 74,4 ng/g of tissue, correspondently. Conclusion: Results of this study allows predict possible additive effects of NSAIDs and anti-estrogens or aromatase inhibitors in prevention and treatment of breast cancer.

176 Expression levels of estrogen receptors -α, -β, -βcx and their associated coregulators during acquisition of tamoxifen resistance in breast cancer.

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The estrogen receptor (ER) antagonist Tamoxifen (TAM) is the treatment of choice for hormone-dependent breast cancer. However, despite an initial response, many patients eventually acquire resistance. Identifying the mechanism (s) by which this occurs is unknown, but is fundamental to preventing relapse and promoting patient survival. The aim of this study was to monitor expression of components of the ER-signalling pathway, namely ERs and their associated co-regulators, during the development of TAM resistance in a cell line model. TAM-resistant cells were developed from the TAM-sensitive MCF-7 cell line by continuous culture in the presence of 4-OH TAM over several months. Cell response to TAM was assessed by MTT assay and RNA extracted for analysis, both on a monthly basis over a 6-month period. Expression of ERα, -β, β cx, the coactivators AIB-1, SRA and SRC-1 and the co-repressors NCoR, REA and SMRT were quantitatively analysed by real-time RT-PCR using the SYBR green method, where differences in target gene expression was relative to that of the housekeeping gene RPLP0. Baseline expression of ERα was greater than ERβ or -β cx. From months 1-6, a gradual loss of expression of ERα was observed, resulting in an overall decrease of 30% by month 6. With ERβ and -β cx a much more dramatic loss of about 60% expression was observed in the first 2 months, after which steady state levels were seen. The co-regulators AIB-1, SRC-1, REA and SRA showed a gradual upregulation, reaching a maximum by 25% over basal values after month 3. No effect on expression of NCoR or SMRT was observed. In conclusion, our results demonstrate that ER subtypes and their respective co-regulatory proteins show subtle but significant changes with the development of TAM resistance in vitro. The early and sustained loss of ERβ and -β cx may be one of the hallmarks associated with this phenomenon. We are currently extending this work in a clinical setting by analysing and comparing expression of the above genes in archival clinical material from patients who developed TAM resistance and those who remained sensitive.

177 The role of estrogen receptor β expression in breast carcinogenesis and progression.

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Background: Estrogen signal transduction plays very important roles in both normal mammary development and neoplastic progression. Since the discovery of estrogen receptor-β (ERβ) there have been many controversial reports on the role of ERβ in breast carcinogenesis and progression, and prognostic implications.

Material and Methods: Using messenger RNA (mRNA) in situ hybridization, we examined ERβ expression in 84 paired normal and cancer tissues, 7 fibroadenoma, and 14 metastatic lymph nodes. We determined the intensity and area (proportion of cells with positive hybridization) of the mRNA hybridization signals and gave scores 0 to 3; no hybridization (0), minimal (1), moderate (2), and strong (3) by the hybridization intensity and no hybridization (0), hybridization in less than 10% of cells (1), 10% - 50% (2), and more than 50% of cells (3) by the proportion of positively hybridized cells. Clinico-pathologic data were available for statistical analysis.

Results: We found no statistical difference in ERβ expression between normal and fibroadenoma tissues but a statistically significant difference between these two and cancer tissues or metastatic lymph nodes ($p< 0.01$). In cases of positive hybridization, the sum of scores of intensity and area were also significantly higher in normal and fibroadenoma tissues than in cancer or metastatic lymph nodes ($p<0.01$). There was no significant correlation between ERβ expression in cancer tissues and the clinico-pathologic data such as patient age, menopausal status, tumor stage, ER or PR expressions.

Discussion: Contrast to ERα, ERβ expression is decreasing in the process of breast cancer development and progression, which suggest the protective role of ERβ in breast carcinogenesis and progression. The relationship between ERβ expression in cancer tissue and other clinico-pathologic factors was certain in the present study. Further investigations are necessary to verify the exact roles of ERβ expression in cancer development, progression and antiestrogen treatment.

178 MDA-MB-231 estrogen receptor beta stable clones exhibit distinct growth and transcriptional characteristics.

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Background: We previously reported stable tranfection of estrogen receptor (ER)α into the ER-negative MDA-MB-231 cells (S30) as a tool to examine the mechanism of action of estrogen and antiestrogens (Jiang and Jordan, *JNCI*, 84:580-591, 1992). To directly examine the mechanism of ERβ action, we have similarly created ERβ stable transfectants in MDA-MB-231 (ERβ 41 cells). **Materials and Methods**: MDA-MB-231 cells were stably transfected with ERβ cDNA and clones were screened by ERE-luciferase assay and ERβ mRNA expression was quantitated by real-time RT-PCR. ERβ 41 cells were compared with S30 cells with respect to their growth properties, ability to activate ERE- and AP-1 luciferase reporter constructs, and the ability to activate the endogenous ER-regulated tranforming growth factor alpha (TGFα) gene. **Results**: ERβ 41 cells express 200-fold higher ERβ mRNA levels compared with untransfected MDA-MB-231cells. Unlike S30 cells, 17-β-estradiol (E2) does not inhibit ERβ 41 cell growth. ERE-luciferase activity is induced 6-fold by E2 whereas neither 4-hydroxytamoxifen (4-OHT) nor ICI 182, 780 actvated an AP-1-luciferase reporter. TGFα mRNA is induced in response to E2, but not in response to 4-OHT. **Discussion**: MDA-MB-231/ERβ clones exhibit distinct characteristics from S30 cells including growth properties and the ability to induce TGFα gene expression. This result is consistent with the known weak AF-1 activity associated with ERβ, and our triple-point hypothesis that AF-1, along with an intact helix 12 and a negative charge at amino acid 351 is required to elicit the agonist activity of 4-OHT with respect to TGFα induction (Liu et al., Cancer Res., 61:3632-39). Furthermore, ERβ, at least in the context of the MDA-MB-231 cellular milieu, does not enhance AP-1 activity in the presence of antiestrogens. In summary, we have successfully established stable MDA-MB-231/ERβ clones that will allow us to more directly compare the functional similarities and differences of ERα and ERβ.

179 Progesterone receptor isoform B induces insulin receptor substrate-2 expression in breast cancer cells.

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Progesterone is involved in breast cancer development and has been proposed to prime breast cancer cells for the actions of growth factors and cytokines. Insulin receptor substrate (IRS) proteins are important components in growth factor and cytokine signaling pathways and are also implicated in breast cancer cell growth. Estrogen has been shown to up-regulate both IRS-1 and -2 expression in breast cancer cells. Previously IRS-2 was reported to be up-regulated in HeLa cells stably transfected with PR-B. In this study we investigated the effect of progestins on IRS-1 and -2 expression in breast cancer cells. Using western blot analysis, we found that IRS-2 protein levels in MCF-7 and T47D breast cancer cells were increased significantly by treatment with the synthetic progestin R5020, while IRS-1 levels were mildly elevated. The IRS-2 level increase was maximal at 24-48 h after R5020 treatment. Optimal induction of IRS-2 expression was observed with concentration of 10-8 M R5020. Anti-progestin Ru486 inhibited the R5020 induction of IRS-2. The transcription inhibitor 5,6-dichlorobenzimidazole riboside and translation inhibitor cycloheximide blocked the IRS-2 induction by progestins, which suggested that the progestin effect on IRS-2 is through a transcriptional mechanism. We also found that PR-B is responsible and required for the progestin effect on IRS-2, by using C4-12 cells (a specifically selected ER and PR negative MCF-7 cells) stably transfected with PR-A and -B. Progestins had no effect on IRS-2 expression in PR-A and non-transfected C4-12 cells. Using immunoprecipitation, we also showed that progestin treatment followed by IGF-I stimulation dramatically increased total tyrosine-phosphorylated IRS-2 level in breast cancer cells. The PI-3 kinase p85 subunit binding to tyrosine-phosphorylated IRS-2 was remarkably enhanced along with the activation of downstream Akt. Our data indicated that progestins differentially regulate IRS-1 and -2 in breast cancer cells, which may have implications in the unique function of progesterone in breast cancer cell growth.

180 Akt alters apoptotic responses to tamoxifen both in vitro and in vivo.

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The Akt kinase is a serine/threonine protein kinase that has been implicated in mediating a variety of biological responses. Studies show that high Akt activity in breast carcinoma is associated with a poor pathophenotype as well as hormone and chemotherapy resistance, including resistance to the antiestrogen, tamoxifen. Akt promotes cell survival by phosphorylating and inactivating proapoptotic proteins and increasing the transcription of survival genes. Akt promotes proliferation and cell cycle progression by activating the mammalian Target of Rapamycin (mTOR), inhibiting the cell cycle inhibitors p27 KIP1 and p21 CIP1 and increasing cyclin D expression. In addition, it has recently been shown that Akt phosphorylates the estrogen receptor a at Ser-167, leading to tumor cell growth and anti-estrogen resistance.

To explore the role that specific components of the Akt kinase pathway play in the cellular response to tamoxifen, we stably transfected MCF-7 cells with an expression plasmid for a constitutively active Akt. We found that MCF-7 breast cancer cell lines expressing a constitutively active Akt are able to proliferate under reduced estrogen conditions, and are resistant to the growth inhibitory effects of tamoxifen, both in vitro as well as in vivo in xenograft models. Initial analysis of the molecular responses in the Akt/MCF-7 xenografts to tamoxifen suggests that high Akt activity alters apoptotic responses to tamoxifen. Control MCF-7 xenografts demonstrated activation of the proapoptotic forkhead (FKHR) transcription factor in response to tamoxifen treatment, while the xenografts expressing the constitutively active Akt transgene demonstrated no alterations in FKHR expression. In addition, TUNEL analysis demonstrated higher levels of apoptosis in the control xenografts in response to tamoxifen treatment compared to the Akt xenografts. Inhibition of Akt activity in vitro restored apoptotic responses to tamoxifen in the Akt/MCF-7 cells to those observed in the control cells. These data suggest that alteration of survival responses is an important mechanism by which Akt confers resistance to tamoxifen.

181 Pattern of epigenetic silencing of genes in human breast cancer: correlation with tumour grade.

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Background: A growing body of evidence now supports the hypothesis that 'epigenetic' mechanisms such as DNA methylation play an important role in neoplasia. The aim of this study was to determine the pattern of expression of multiple key cancer genes that are known to undergo epigenetic inactivation by promoter hypermethylation in breast cancer.

Methods: The expression of the tumour suppressor gene p16, estrogen receptor (ER)a, ERb, progesterone receptor (PR) and the DNA repair genes hMLH1 and MGMT were studied with immunohistochemistry in 200 breast cancers. Methylation specific PCR was performed in a subset of 20 cancers to confirm the role of DNA methylation in the loss of expression of MGMT.

Results: 78.5 % of breast cancers showed loss of expression of at least one gene (n = 157). A strong correlation was seen between high grade tumours and loss of expression of ERa ($p < 0.001$), PR ($p < 0.001$), ERb ($p < 0.001$) and MGMT ($p = 0.04$), while loss of expression of p16 was associated with low/intermediate grade tumours. No loss of expression of hMLH1 was seen in any of the tumours studied. DNA methylation was strongly associated with loss of expression of MGMT ($p<0.001$).

Conclusions: High grade breast tumours showed methylation-linked silencing of several key cancer genes and therefore can be targeted for therapeutic strategies based on epigenetic events such as demethylating agents and histone deacetylase inhibitors.

182 Breast cancer functional genomics.

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Breast cancer is a heterogeneous and dynamic disease. This heterogeneity is most probably based on the acquisition or loss of several characteristics; 1- Self sufficiency in proliferative growths signals, 2- Loss of differentiation, 3- Insensitivity to growth inhibitory signals, 4- Evasion of apoptosis, 5- Acquisition of independent replicative potential, 6- Induction of angiogenesis, 7- Induction of invasion and metastasis, 8- Capability of developing drug resistance.

The use of microarray technology in cancer research is without any doubt the most comprehensive way to deal with multigenic and heterogeious disease. In an effort to dissect the pathways and mechanisms involved in this complex multistep process and avoid system variability, we have developed a breast cancer comprehensive database that can help interpret, prioritize and validate some of the tumor profiling data even for genes whose function is not yet known. The breast comprehensive database consist of seven databases that deals with In Vivo tumor profiling, proliferation, Drug sensitivity and resistance, Hormonal impact, Apoptosis, epigenetic and post-translational regulation of differentiation. All the databases were generated using the same experimental protocol and the same cDNA arrays consisting of 14.000 human known genes. A databases for the most important genes is annotated with information from other in silico databases such as LocusLink, Signal peptide, SAGE... This database serve as a link between tumors clinical data, functional assays on cell lines and on-line databases as a mean to prioritize and speed-up the search for genes that may be of critical value as diagnostic, prognostic or a drug target.

183 Loss of heterozygosity in normal tissue adjacent to primary breast carcinomas.

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Background

Morphologically normal cells adjacent to breast carcinomas are known to harbor genetic abnormalities commonly observed in malignant tissues. It is unclear whether genetic changes in adjacent normal cells reflect random mutational events or coordinated patterns of genetic change characteristic of the neighboring tumors.

Material and Methods

In this study, laser capture microdissection of paraffin embedded breast tissue samples was used to define patterns of loss of heterozygosity (LOH) in different classes of breast cancer, including ductal carcinomas in situ, lobular carcinomas in situ, papillary carcinomas, and infiltrating ductal carcinomas, as well as in surrounding morphologically normal stromal and epithelial cells. High-throughput fluorescent genotyping of 52 anonymous microsatellite markers was conducted to survey 26 chromosomal regions commonly deleted in breast cancer.

Results and Discussion

In situ carcinomas showed rates of LOH greater than those in more advanced invasive tumors, and a higher prevalence of LOH was observed in normal cells adjacent to in situ carcinomas (63%) than in cells adjacent to invasive lesions (38%). LOH in adjacent normal cells was most prevalent on chromosomes 2, 3, 5, 6, and 13. Interestingly, patterns of LOH in normal stromal and epithelial cells differed from those in the neighboring tumors in ~50% of the cases examined. Patterns of chromosomal alterations in normal tissue adjacent to breast carcinomas may identify molecular events underlying the early stages of breast cancer development and help define tissues prone to cancer recurrence.

184 Identification of genes regulated by the RXR-selective retinoid, LGD1069, in human breast cells using oligonucleotide arrays.

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Retinoids have been found to be promising chemopreventive agents that play an important role in regulating cell growth, differentiation and apoptosis of many different type cells. The action of retinoids is mediated by nuclear receptors, including retinoic acid receptors (RARs) and retinoid "X" receptors (RXRs). These nuclear receptors are transcription factors that bind retinoids and regulate gene expression. LGD1069 is a highly selective RXR agonist that has reduced toxicity compared with other nonselective retinoids. Our previous studies have demonstrated that RXR-selective retinoids, including LGD1069, inhibit the growth of normal and malignant breast cells and suppress the development of breast cancer in transgenic mice with no apparent toxicity. In this study we have identified biomarkers of the chemopreventive effect of this RXR-selective retinoid using microarrays. We used oligonucleotide-based microarrays to identify the early or late downstream targets of LGD1069 in normal human breast cells. Normal breast cells were treated with vehicle or LGD1069 for 1hr and 24hr, and then RNA was harvested. Biotin-labeled transcripts were then prepared and used to hybridize to Affymetrix arrays. Analysis of the results was performed using Affymetrix and dChip analysis programs. These results identified more than 100 genes up-regulated and down-regulated by LGD1069. We have investigated 20 of these genes, and have validated that LGD1069 modulates their expression using quantitative RT-PCR and Western blotting. Genes found to be regulated by LGD1069 include known retinoid-regulated genes (SCD-1, and RARβ), growth regulatory genes (IGFBP6, Id-1, COX-2, ODC, and cMet), and differentiation markers (keratin 15, and 17). We are currently studying these genes to determine whether they are regulated *in vivo* using mammary gland tissue from transgenic mice treated with LGD1069. These critical growth regulating proteins will be promising targets for future agents for the prevention or treatment of breast cancer.

185 Molecular classification of breast cancer patients by gene expression profiling.

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Background: For many tumors pathological subclasses exist which have to be further defined by genetic markers to improve therapy and follow up strategies.

Material and Methods: We have performed cDNA array analyses of breast cancers to classify tumors into categories based on expression patterns.

Results: By cluster analysis a subpopulation of breast cancers was identified, which contained a high number of nodal positive tumors and frequently had developed distant metastases at the time of diagnosis. Correlation of follow up data with this cluster analysis revealed that despite a relative short follow up time already 50 % of the patients in the identified subpopulation progressed to metastatic disease. Strikingly, three of five patients classified as N0 in this subpopulation developed distant metastases.

Discussion: Taken together, the use of these differentially expressed marker genes in conjunction with sample clustering algorithms provides a novel molecular classification of breast cancer specimens, which facilitates the identification of patients with a higher risk of recurrence.

186 Phase I, open-label, randomized, crossover study of the pharmacokinetic interaction of toremifene and atamestane (Biomed 777) in healthy postmenopausal women.

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Background: Atamestane (Biomed 777) is a potent and selective steroidal type I aromatase inactivator (AI) selectively interrupting the final step of estrogen biosynthesis. Toremifene is a nonsteroidal triphenylic estrogen receptor blocker. At the labeled dose of 60 mg/day p.o., toremifene has been shown have a lower intrinsic estrogenic agonist activity than tamoxifen in vivo. The goal of combining atamestane with toremifene is to slow disease progression more effectively than monotherapy with either compound. Because the AI letrozole had shown an adverse interaction with tamoxifen, we performed this pharmacokinetic (PK) interaction study in preparation for phase 3 trials.

Material and Methods: Fourteen healthy postmenopausal subjects were enrolled in an open-label, randomized, three-way crossover study. Each subject received 7 days of treatment, separated by 28 days, of either a) toremifene alone 60 mg a.m., b) atamestane alone 300 mg a.m. and 200 mg p.m. or c) atamestane 300 mg plus toremifene 60 mg a.m. followed by atamestane 200 mg p.m.. Safety labs were measured at screening, days 1, 7 and end of study. Plasma concentrations of both compounds were determined following the final dose of each treatment period (days 1, 7-10 with atamestane and on days 12,17,28, 35 during toremifene dosing).

Results: Twelve patients completed the full study period. No drug-related serious adverse events or unexpected side effects were observed.

Ratios of AUC and Cmax for Single vs Combined Drug Administration

Compound	Ratio	Parameter	Mean Ratio	Lower 95% CI	Upper 95% CI
Atamestane	A/A+T	AUC (0-t) (ng.h/ml)	0.85	0.40	1.80
		Cmax (ng/ml)	0.87	0.31	1.80
Toremifene	T/A+T	AUC (0-24) (ng.h/ml)	1.03	1.00	1.07
		Cmax (ng/ml)	0.99	0.87	1.12

Analyses were performed on log transformed PK indices

Conclusion: No relevant PK interaction was observed between atamestane and toremifene at clinically recommended doses. No adverse effects on tolerability or clinical laboratory values were observed. This is the first formal demonstration of non-interaction between these two classes of drugs, suggesting that certain combinations may be more favorable than others.

187 New progesterone receptor antagonists inhibit growth of human breast cancer xenografts and prevent carcinogen induced breast cancer in rats by modes of action different from other hormonal agents.

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The biological activity of progesterone is mediated by the progesterone receptor (PR), which induces a cascade of transcriptional events, critical for maintenance and development of female reproductive organs. Blocking PR function by using a PR-antagonist allows the modulation of various endocrine processes which also might be responsible for gynecological or oncological diseases.

It is well known that progesterone, in physiological concentrations participate in the proliferation of mammary carcinomas. Therefore it is obvious that antiprogestins can block the growth of breast tumors functionally expressing the PR.

We describe the pharmacological characterization of a novel, highly potent PR-antagonist, that has a considerable potential for therapeutic intervention in breast cancer. The PR-antagonist showed high antiprogestagenic activity in vitro on both PR isoforms PR-A and PR-B. This high antiprogestagenic activity could also be demonstrated in several in vivo models. Subsequent experiments with breast cancer models showed a strong antiproliferative activity. In the nitroso-methylurea (NMU) and dimethyl-benzanthracene (DMBA)-induced mammary tumor models in the rat, treatment with the PR-antagonist completely suppressed the growth of established tumors and prevented the development of breast tumors when given prophylactic. Previous studies on breast tumors treated with an antiprogestin have indicated that the growth inhibitory effect is accompanied by the initiation of terminal differentiation leading to apoptotic cell death (Michna et al. J Steroid Biochem Mol Biol 1992;41:339-348). Induction of tumor cell apoptosis was also found in our studies. The ability of these compounds to induce tumor cell differentiation that leads to apoptosis is unique among all other endocrine therapeutics. Our results revealed that the biological response to a progesterone antagonist does not seem to be only the result of competition of progesterone but rather may be accompanied by additional mechanisms.

With these pharmacological properties a PR-antagonist may be a promising new option for clinical breast cancer therapy.

188 Breast cancer risk factors among rural Terena Indian women, state of Mato Grosso do Sul, Brazil.

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Background: Breast cancer, the leading cause of cancer in the general population of the State of Mato Grosso do Sul (MS), has not been reported by the national health system among Terena Indian women living in native communities, despite the fact that cancer at other sites (cervix, ovarian, lung, abdominal tumors, among others) have indeed been diagnosed.

Material and Methods: Aiming to evaluate the distribution of selected breast cancer risk factors among Terena Indian women in MSl, a survey was carried out and two samples were interviewed , respectively, 330 voluntary women from ten rural Indian villages and 40 women from Limao Verde village. Reproductive life patterns, diet characteristics, familial and personal medical antecedents were traced, and body mass index was evaluated.

Results: Menarche mean age at 12.3 yr. old (30% at 13 yr. old or later), one or more pregnancies reported by 86% of women (42% reporting 5 or more), mean reported parity of 4.6 children per woman, first pregnancy mean age at 18.9 yr., (3.8% after 28 yr. old), lifelong total breastfeeding mean during 84 months, and 70% reporting menopause under 50 yr. old were observed in the larger sample. Dietary pattern revealed a high intake of fruits, vegetables, pasta and roots, and scarce intake of red meat or chicken. These patterns were quite similar in the smaller sample, in which overweight (BMI >24) was observed among 77 % of women. Alcohol intake was unreported, and physical activity is not usually carried out by women in these communities.

Discussion: As a whole, these results indicate a very different pattern of breast cancer risk factors comparatively to the Brazilian general population. Mean late age at menarche, large parity followed by lengthy breastfeeding and early menopause possibly indicate shorter lifelong estrogen stimulation than usually observed in the Brazilian general population. All these, but exercise fitness profile, reveal a pattern of low risk to breast cancer among the analyzed samples of Terena rural women in MS which maybe is associated to the absence of breast cancer reports among them. Continuous migration of these communities members to urban areas, and adoption of a new lifestyle pattern may modify this breast cancer risk factors profile in a near future.

201 Delayed fixation results in a rapid decrease of phosphorylated HER2/ErbB-2 detection by IHC in EGF-stimulated A431 cells.

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Background: The phosphorylated-HER2/ErbB-2 receptor (phospho-HER2) has been shown to provide valuable prognostic information for human breast cancers. However, formalin fixation of clinical specimens may be delayed after surgical removal, and phosphoproteins are known to dephosphorylate rapidly *in vitro*. In the present study, a human breast carcinoma cell line, A431 cells, was utilized to investigate the effect of delayed fixation on the detection of phospho-HER2 by immunohistochemistry (IHC).

Materials and Methods: EGF was added to 1 of 2 culture flasks containing A431 cells such that the final concentration was 500 ng/ml. An equal volume of EGF-free vehicle was added to the second flask as a baseline control. The cells were incubated in EGF (or vehicle) for 40 minutes. Then, the cells were washed 3 times in cold PBS and suspended in PBS at room temperature. At 0, 2, 5, 24, and 48 hours, aliquots of cells were removed, fixed in 10% neutral buffered formalin and processed into paraffin blocks. IHC analysis of paraffin sections was performed using an anti-phospho-HER2/Tyr1248 antibody (Cell Signaling Technology) and DAB visualization following heat-induced epitope retrieval. The staining intensity and percentage of positively staining cells were scored semi-quantitatively by a pathologist and converted to H-scores for analysis.

Results: The results showed an exponential decrease in the phospho-HER2 H-scores of EGF-stimulated A431 cells versus time. The phospho-HER2 signal was significantly decreased ($p<0.05$) at 2 hours versus 0 hours. At 5 hours, the phospho-HER2 score had decreased to 24% of the maximum value and at 24 hours, the score had decreased to 1.6% of maximum. Phospho-HER2 could not be detected in the baseline control cells, nor in the stimulated cells after 48 hours.

Discussion: The results show that phospho-HER2 detection by IHC is highly dependent on the time period between the removal of EGF-stimulated A431 cells from culture and addition to fixative. This suggests that the activated state of the HER2 receptor is not stable *in vitro* and that phospho-HER2 detection by IHC may not adequately reflect the *in vivo* condition in clinical specimens where the time to fixation is not well controlled.

202 Assessment of Her2-neu status in core needle biopsy of 215 patients with primary breast cancer.

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Background: Her2-neu overexpression in breast cancer patients is associated with worse prognosis and resistance or sensitivity to specific treatment. Core needle biopsy is an easy and cost-effective method to obtain pretherapeutically information about tumorbiology and cancer specific markers. The aim of our study was to investigate the accuracy of her2-neu assessment in core needle biopsy tissue.

Material and Methods: Her2-neu status was evaluated by immunhistochemistry (HercepTest™) of formalin-fixed, paraffin embedded biopsy tissue and surgical removed tissue in 215 patients, 72pat. (33,5%) received preoperative chemotherapy, 143pat. (64,5%) had no preoperative treatment. Intensity of staining was scored according to the guidelines of HercepTest™ (neg, 1+, 2++, 3+++). Fluoreszenz in situ hybridization (FISH) was performed in all patients with immunhistochemically 3+ positive core needle biopsies. We calculated the accuracy, sensitivity, specifity and Kappa coeffizient of her2-neu results and correlated strong positive tumors versus negative, 1+ and 2++.

Results: Her2-neu overexpression was found in core needle biopsies of 26pat.(18.2%) in the preoperative untreated group and in 14pat.(22%) in the preoperative chemotherapy group.

Sensitivity of Hercep test in core needle biopsy and final pathological specimen was 69%, specifity 97%, accuracy 92% and simple Kappa coeffizient was 0.72 in the preoperative untreated group. Based on this finding we performed FISH analysis in 3+positive patients. Accuracy of the combined analysis of her2-neu status was increased to 97% with a sensitivity and specifity of 95% and 98% respectively in the preoperatively untreated group. The preoperative chemotherapy group did show slightly worse results - sensitivity was 91%, specifity 92%, accuracy 92% by means of the combined analysis.

Discussion: The assessment of her2-neu status by core needle biopsy in breast cancer is feasible. Immunhistochemical strong positive results should be proven by FISH in order to minimize the number of false positive results by immunhistochemistry alone. Preoperative treatment with trastuzumab can be based on these results.

203 Histology of CerbB2 positive breast carcinoma; does lymphoid infiltration predict tumor behavior?

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Background: Most breast carcinomas are infiltrated by variable numbers of lymphocytes and plasma cells. When this infiltrate is prominent, as in medullary carcinomas, the infiltrates frequently exhibit markers of immune response to tumor antigens and patients have better prognossis. We have found in routine examination of breast tumors that a portion of Her-2 positive tumors have prominent lymphoid infiltrates. We hypothesize that prominent lymphoid infiltrates in CerbB2+ breast cancers may identify tumors that may behave differently from tumors without prominent lymphoid infiltrate.

Materials and Methods: Tumors over-expressing CerbB2 antigen and grade 3 mammary carcinomas [controls] were identified from pathology records. Routine H&E tissue sections were reviewed for the presence of lymphoid infiltrates. Grade, stage, estrogen and progesterone activity, p53 determination and CerbB2 status were also recorded. We compared tumor morphologic features to grade, stage, marker studies, and disease status to determine if the presence of inflammation was related to prognosis.

Results: 130 CerbB2+ tumors and 47 grade 3 CerbB2 neg tumors were identified. 80 tumors had p53 mutations (p53+) and 116 were estrogen receptor positive(ER+). 25 developed distant disease (DD) 1 to 4 years following therapy. 71 tumors had focal or no lymphoid infiltrates(0), 66 had patchy infiltrates (1+) and 40 had heavy infiltration(2+). 71 tumors had lymph node metastases (LNM). Comparison of all tumors showed a significant association of lymphoid infiltrates with ERneg ($p < 0.001$), p53+ ($p < 0.01$), Grade 3 tumors ($p < 0.001$), and advanced disease (LNM+DD) ($p< 0.05$), but not to Cerb-B2+, LNM, DD, or primary tumor stage. For CerbB2+ tumors, p53 mutation ($p< 0.0001$) and advanced disease ($p< 0.05$) were associated with increased lymphoid infiltrate.

Discussion: Lymphoid infiltration of breast cancers is associated with ERneg, p53+, high tumor grade, and advanced disease, but does not appear to be associated with CerbB2 positivity. Lymphoid infiltration in CerbB2+ tumors is associated with p53 mutation and advanced disease, but does not appear to predict development of distant disease.

204 Molecular characterization of mammary ductal epithelial cells using interphase fluorescence in-situ hybridization (FISH).

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Background: Increasing usage of technologies that sample mammary ductal epithelial cells has placed substantial emphasis on cytologic interpretation. It has been demonstrated that genetic abnormalities may be present in ductal epithelial cells prior to morphologic changes seen on cytologic examination. We hypothesized that characterization of chromosomal abnormalities by FISH may provide objective data to confirm cytologic interpretation. Methods: Cytology slides from patients who underwent ductal washings obtained either by microcatheter or mammary ductoscope were used as the basis for the study. Ductal washings were collected prospectively and processed using ThinPrep, and slides were interpreted by an experienced cytopathologist. The identical slides were destained and four-color interphase FISH was performed using peri-centromeric probes for chromosomes 1, 8, 11 and 17. Aneusomy was defined as chromosomal loss or gain in at least 2 non-overlapping ductal cells, and a separate pathologist who was blinded to the cytologic interpretation performed FISH analysis. Results: A total of 25 cytology slides have been analyzed by FISH thus far. 4/4 of the slides that were interpreted as containing malignant cells by cytopathology demonstrated gains of chromosomes 1, 8, 11 and 17. By contrast, 6/6 of the slides that were determined to be benign by cytology demonstrated no cells with chromosomal gains, although 5/6 had cells that were monosomic for chromosome 17. Of the remaining 15 slides that were interpreted as atypical, 4/15 demonstrated no chromosomal abnormalities while 5/15 had multiple chromosomal gains. Conclusions: These data demonstrate that ductal epithelial cells can be analyzed using FISH directly on the ThinPrep slides. Importantly, in those specimens that were interpreted as benign or malignant by cytology, chromosomal gains detected by FISH analysis appeared to correlate with the morphologic diagnosis. Chromosomal gains in the cytologically non-diagnostic atypical cells may be of prognostic value and provides the rationale for use of FISH in prospective clinical trials of ductal lavage.

205 Does increased sectioning of ductal carcinoma *in-situ* (DCIS) pathologic specimens have clinical consequences?

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Background: DCIS often presents as an asymptomatic mammographic finding requiring excisional biopsy. The gross specimen is sectioned for microscopic analysis. There are no consensus guidelines for the processing of excisional biopsy specimens. Increased sectioning may potentially increase the risk of a positive margin. It was our impression that there has been an increase in the number of slides for DCIS specimens at our institution over the last decade. We herein assess the degree of sectioning over time and consider possible clinical consequences.

Material and Methods: Pathologic data from patients who underwent initial excisional biopsies at DUMC and were referred to Radiation Oncology with DCIS from 1992-2002 were retrospectively reviewed. Patients with palpable masses, less than the whole specimen submitted for sectioning, or invasive cancer, were excluded (144/480 DCIS consults were eligible for review). Analysis included: specimen size, number of slides, their ratio, margin status, and whether a re-excision was performed. Mean values were compared utilizing the student's t-test.

Results: See table

Discussion: The number of slides per specimen length increased over time. Despite more thorough microscopic evaluation, the rate of positive margins actually declined, apparently due to larger excisional volumes and/or superimposed changes in tissue fixation/processing. Larger volumes are partly explained by an increased use of pre-excision diagnostic stereotactic biopsies (STNB) and, thus, a higher ratio of therapeutic to diagnostic excisions in the latter years. However, despite a more stable rate of STNB recently, progressive sectioning has continued (98-00 vs. 01-02, $p<0.001$). Increased sectioning may raise the cost without a clear clinical benefit.

Results:

Years	Number of Patients	Mean specimen volume (cm^3)	Mean maximum length (cm)	Slides/Max length (mean, slides/cm)	Positive Margins	Stereotactic Biopsy rate
1992-94	23	49	5.1	2.5	52%	4%
1995-97	28	51	5.6	2.7	46%	4%
1998-2000	69	77	6.7	3.9	23%	62%
2001-2002	24	90	6.9	5.8	25%	75%
92-97 vs. 98-02		p=0.003	p=0.001	p<0.001		

206 Computer assisted complete three-dimensional reconstruction of multiple breast carcinomas. Pathological analysis on discrimination between multicentric and multifocal tumors.

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Introduction: An unanswered, important question concerning multiple breast carcinomas is whether they arise independently in the breast (multicentric) or have multiple invasive tumor development connected by extensive intraductal tumor spread from a single, primary carcinoma (multifocal). We have already reported the first computer assisted, complete three-dimensional (3D) reconstruction of the whole mammary ductal/lobular systems (MDSL) by applying stereomicroscopic techniques (Cancer 2001; 91: 2263-72). In this study, we will report anatomical characteristics of multiple breast carcinomas using our 3D techniques.

Materials and methods: A whole breast obtained from a 44 year-old woman who underwent mastectomy for multiple carcinomas was used in this study. Contours of all ducts of serial 2 mm-thick sections were examined in stereomicroscopic techniques and reconstructed using a computer graphic system (TRI/3D-SRF; RATOC, Tokyo, Japan).

Results: The whole breast consisted of 16 MDLS. Ductal anastomoses were observed at 7 sites in the breast. Three (42.9%) of 7 ductal anastomoses connected different MDSL and were situated less than 2 cm from the nipple. The remaining 4 ductal anastomoses existed in the same MDLS. In this case, all the multiple invasive tumor foci developed over three MDLS continuously connected by extensive intraductal spread of carcinoma through two ductal anastomoses communicating with other MDLS.

Conclusion: The hypothesis was found that multiple invasive tumor foci of the breast did not arise independently, and all the independent tumor foci were connected continuously by extensive intraductal spread of carcinoma via ductal anastomoses. Therefore, it is concluded that multiple breast carcinomas are multifocal and not multicentric in origin, and ductal anastomoses in the MDLS can be possible routes of extensive intraductal spread of carcinoma.

207 Predictors of residual disease in re-excisions for lumpectomies with close margins (less than 0.1 cm).

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Background: Re-excisions (RES) performed for transected or close margins often do not contain residual disease (RD). This study seeks to identify factors that may predict the presence of RD in RES following an initial breast sparing excision procedure in which the margins of resection are free of tumor but in which tumor is very close to the margins.

Methods: 91 lumpectomies with close (less than 0.1 cm clearance), but not transected margins from 1994-1999 and their subsequent RES were analyzed for tumor type near margin, presence of RD, type of RD, stage of infiltrating carcinoma (IC), extent of duct carcinoma in-situ (DCIS), and tumor grades.

Results: 17 of 91 cases were DCIS only; 15 were IC only; and 59 were combined IC and DCIS. 34 cases (37%) contained RD in their subsequent RES while 57 (63%) did not. For the 34 cases with RD, 16 were of T1ab tumor, 7 were T1c, 3 were T2, and 8 were T0 (DCIS only). 61% (22) of RES of cases with extensive DCIS had RD whereas 22% (12) of RES of cases with no DCIS or minor DCIS (less than 1 CM) had RD (Chi-square = 14.35, $P< 0.01$). DCIS was within 0.1 cm of the margin in 53 cases, of these RD was present in 25 (47%) whereas 9 of 38 (24%) with only IC close to the margin had RD ($p < 0.05$). 24 of 50 cases of T0&1ab carcinomas had RD and 10 of 41 T1c&2 tumors had RD ($p < 0.05$). DCIS and IC grades were not related to presence of residual disease.

Conclusions: RD was present in 37% of RES with close but not transected margins in this study. The extent of DCIS in the primary specimen best correlated with the presence of RD in the RES. Type of tumor near margin and size of IC were also, but less significantly, associated with residual disease.

208 Optimization of tumor measurement and surgical margin evaluation in mastectomy and lumpectomy specimens.

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Background : The most important measure of prognosis in breast carcinoma is tumor size. Currently, breast specimens are sectioned free hand utilizing a scalpel or a knife. Since it is difficult to cut the breast in slices of uniform thickness, the estimation of tumor size may be inaccurate. The aim of this study was to assess if the determination of tumor size and surgical margin status would be improved, if surgically resected breast specimens were processed with the aid of a tissue slicer, sectioning the specimen uniformly at 3-mm intervals.

Methods : A total of 20 mastectomy and 10 lumpectomy specimens were used. On receipt, the specimens were weighed, measured, inked and frozen at -20 ° C for 30 minutes to 2 hours depending on specimen size. A tissue slicer (Berkel) was used to serially section the specimen at 3-mm intervals. Accurate measurement of tumor size in three dimensions, distance from the closest surgical margin and evaluation of remaining uninvolved breast tissue was determined.

Results : The use of the tissue slicer allowed us to: 1) accurately measure the tumor size in three dimensions, 2) optimize the assessment of the distance between tumor and the closest margin of resection, 3) more thoroughly examine the uninvolved breast tissue, 4) provide thinner slices allowing for shorter fixation time, smoother cutting of paraffin embedded fatty tissue, and higher quality H&E stained slides, 5) allow greater tissue availability to perform ancillary studies, especially from small breast lesions. Thin slicing allowed mirror image tissue samples to be submitted for routine fixation as well as snap freezing. As a validation of this method, mRNA was successfully isolated from the snap frozen samples.

Conclusions : The use of a tissue slicer improves the ability to perform a more thorough examination of mastectomy and lumpectomy specimens. It optimizes the assessment of tumor size in three dimensions, and surgical margin status. Examination of the remaining breast tissue with preservation of orientation allows for the detection of multifocal and multicentric lesions as well as non-neoplastic pathology. The quality of the H&E stained slides is better, there is no significant freezing artifact, the freezing process does not affect immunohistochemical studies and protects mRNA from being degraded, allowing further isolation for possible future studies.

209 Similar pathologic changes are seen following interstitial laser therapy and radiofrequency ablation of T1 breast carcinomas.

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Background: Interstitial laser therapy (ILT) and radiofrequency ablation (RF) are new techniques for destroying tumor cells in-situ. Prior studies have shown that complete cell death, as assessed by NADH-diaphorase staining, is achieved in the treated area.

Material and Methods : Fifty-four women with localized, mammographically detectable, T1 breast cancers consented to be treated with ILT and six women consented to be treated with RF ablation. Prior to ablation, the diagnosis of breast carcinoma was established by image guided core biopsy. The treated tumors were then excised 5 to 42 days following the ablation.

Results: The ILT and RF treated tumors showed similar histologic changes. The tissue immediately adjacent to the energy source appeared coagulated and showed the same "wind-swept" appearance of nuclei as seen with cautery artifact. Recognizable tumor was present in the adjacent zone and showed no sign of necrosis, increased apoptosis or inflammatory infiltrate on H&E sections. However, the tumor in this zone failed to immunostain for cytokeratin 8/18 despite intense staining in the epithelial cells outside the treated area. Adjacent to this "pseudoviable" zone was histologically necrotic tumor which was in turn surrounded by vascular proliferation and fat necrosis. The breast tissue outside the zone of fat necrosis appeared to be unaffected by the ablation therapy.

Conclusion: ILT and RF ablation show similar morphologic features. It is important for the pathologist to recognize the changes seen following ILT and RF ablation, especially the zone containing "pseudoviable" tumor. Cytokeratin 8/18 may be a marker to identify these likely non-viable but normal appearing tumor cells. The extent of the treated area appears to be defined by vascular proliferation and fat necrosis.

210 Diagnostic and therapeutic potentials for the interaction of a xeroderma pigmentosum G related peptide and an isoform of proliferating cell nuclear antigen in breast cancer cells.

Liu Y, Tomic D, Schnaper L, Hickey RJ, Malkas LH. Indiana University Medical School, Indianapolis, IN; Greater Baltimore Medical Center, Baltimore, MD

Proliferating cell nuclear antigen (PCNA) plays an important role in the process of DNA replication. In conjunction with an adaptor protein, replication factor C (RFC), PCNA forms a moving clamp that is the docking point for DNA polymerases delta and epsilon. Work from another laboratory (Gary et al. 1998) demonstrated that PCNA interacts with a specific domain within the xeroderma pigmentosum G protein (XPG). Xeroderma pigmentosum is a disease that results from a recessive genetic defect rendering individuals sensitive to the damaging effects of ultraviolet light. Our laboratory observed the expression of two iso-forms of PCNA in malignant cells, and our own efforts to purify the individual forms of PCNA led to the discovery that a fragment of the XPG protein can bind selectively to the unique form of PCNA expressed only by cancer cells. Here we report the development of an enzyme-linked immunosorbent (ELISA) assay to distinguish the PCNA iso-form specific to cancer cells (csPCNA) from the PCNA in non-malignant cells (nmPCNA) using a biotin labeled XPG fragment containing the PCNA binding domain. The biotinylated XPG fragment presents much stronger binding affinity to csPCNA in MCF7 breast cancer cells when compared to the binding with nmPCNA in MCF10A primary breast cells. Normal and breast cancer tissues from patients were also examined to determine the relationship of csPCNA expression and cancer progression. The effects of overexpression of this XPG fragment on cell proliferation was also evaluated in both primary breast cells and breast cancer cells. This is the first time that a simple ELISA assay targeting PCNA could potentially serve as a marker for cancer diagnosis. Its potential in cancer therapy will also be described.

211 Assessment of microvessel density in core biopsy specimen in relation to tumour size in breast cancer.

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Background: Core biopsy is a simple and useful diagnostic tool in breast cancer and may be the only specimen available for analysis of predictive and prognostic factors in patients undergoing primary systemic treatment. Recent data indicate that angiogenic activity in primary breast cancer may be helpful in predicting response to given therapy.

Material and methods: During 1999-2001 angiogenesis was evaluated in 54 consecutive patients with immunohistochemical staining and calculation of microvessel density (MVD) in core biopsy specimen as well as in excised tumours. MVD was scored both as a continous variable and a dichotomous variable. Patients receiving preoperative treatment or only given medical treatment were not included. Four specimen were excluded due to failure of staining.

Results: MVD in tumour specimen was 18-62, median 33, and in core biopsy specimen 18-60, median 32.No significant correlation was identified between them However, for tumours larger than 20mm (n=23) a significant correlation was noted (rr=0.56, p=0.005) as well as for tumours larger than 30mm (n=14, rr=0.71, p=0.004) between MVD in tumour specimen and core biopsy specimen. Lobular cancers (n=19) constituted most of the large tumours and for this histopathological subgroup a significant correlation (rr=0.55, p=0.014) was noted. The results were identical when scoring MVD as a dichotomous variable using chi-square-analysis.

Conclusion: Angiogenic activity in breast cancer can be assessed with MVD-scoring in core biopsy specimen from tumours larger than 20mm. In the clinical setting when preoperative treatment is optional most tumours are larger than 20mm.The above data indicate that angiogenesis may be added to other prognostice and predictive factors that can be estimated in core biopsy specimen and provide useful information before systemic treatment is given.

212 Prospective study on the prognostic impact of minimal residual disease in bone marrow of breast cancer patients.

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Introduction: Most of the current prognostic factors in breast cancer have a clinical impact. However, their prognostic estimate for individual patients is not sufficient. Therefore additional new prognostic factors have to be identified and considered in therapeutic decisions. Especially breast cancer patients with a local curative resection (R0) and without solid metastases (M0) at risk for early relapse have to be identified prospectively.

Aim of the study: Evaluation of the prognostic impact of the BM-status - defined as immunocytochemically detected epithelial cells in bone marrow - in breast cancer patients (R0M0) as indicator for a systemic disease at primary diagnosis.

Methods: Bone marrow aspirates of breast cancer patients (n=1045) were analyzed in a standardized procedure (1 million cells per patient; APAAP-staining protocol; MoAb CK2). Statistic evaluation included correlation of the BM-status with classic clinico-pathological parameters and overall survival of the patients (Kaplan-Meier test; Cox Hazard regression analyses).

Results: A positive BM-status can be detected in 27.1% (283/1045) of the primary breast cancer patients and does not correlate with age and classic clinico-pathological factors (i.e. pT-stage, pN-stage, grading, histological type, hormone receptor status and tumor markers CEA / CA15-3). The BM-status is prognostically relevant in breast cancer patients (stage R0M0). A positive BM-status correlates with shorter overall survival after a median follow-up of 52 months (range 6-120) in univariate analyses (p=0.006). Furthermore the positive BM-status was identified as an independent prognostic factor in multivariate analyses (RR 1.41; 95%CI 1.02-2.00; p=0.049). In the strongest Cox regression based on a clinically relevant dichotomisation the positive BM-status ranges behind the axillary lymph node status (RR 3.53; 95%CI 2.37-5.26; p=0.0001), the hormone receptor status (RR 2.63; 95%CI 1.86-3.71; p=0.0001) and the pT-stage.

Conclusion: The present prospective study demonstrates an independent prognostic impact of the BM-status on overall survival of breast cancer patients and should therefore be considered in future Consensus statements for the adjuvant therapy of breast cancer patients.

213 Clinical implication of detection of isolated tumor cells in bone marrow in early breast cancer. Results from the Oslo study.

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Background: Detection of tumor cells (TC) in bone marrow (BM) in breast cancer (BrCa) can potentially be used for prognostication, therapy monitoring and characterisation for individualised treatment. The presence of tumor cells (TC) has been shown to affect clinical outcome. However, the clinical significance of TC detection in node negative (N0) differs in previous studies. The aim of this study is to clarify the clinical impact of detection of TC in BM in both node positive(N1) and N0.

Material and Methods: BM aspirates were collected during operation from 920 patients (pts) with early BrCa. Mononuclear cells were isolated, cytospins prepared, followed by immunocytochemical analysis (using the anticytokeratine antibodies AE1 and AE3) and detection of TC. Immunohistochemical analysis of erbB2, p53, catepsinD, ER and PgR-expression were also performed. All pts received therapy according to the National Guidelines. The pts were reexamined every 6-12 months.

Results: 532 pts with N0 (63%) and 288 pts with N1 status (34%) were enrolled and evaluable (total 848 infiltrating carcinoma). TC were detected in BM in 12.6% (N0 9.4%, N1 19.5%). Median FU is 49 months. So far 189 pts have experienced a relapse, 87 locoregional and 133 systemic. BrCa death has ocurred in 100. No difference in the frequency of locoregional relapse was observed in BM+ vs BM- pts, whereas 28.8% of BM+ (N0 8.0%, N1 24.0%) and 13.1% of BM- (N0 7.8%, N1 49.1%) experienced systemic relapse. BrCa deaths were detected in 43.9% of the BM+ and 18.9% of the BM- N1 pts (p=0.003, logrank test). No difference in survival was observed in N0 pts.

Conclusion: Detection of TC in BM predicts future systemic relapse and BrCa death in N1 BrCa. However, based on this study, BM status cannot be used as a prognostic factor for N0.The primary tumor analyses will be compared to the BM results and clinical outcome - and presented.

214 Detection and monitoring of circulating tumor cells (CTCs) by CK19 mRNA expression in stage II/III breast cancer patients treated on a propective, randomized clinical trial of sequential neoadjuvant chemotherapy.

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PURPOSE: To evaluate the feasibility of detecting CTCs by CK19 mRNA in early stage breast cancer patients and determine the ability of this marker to predict response to therapy and recurrence.

PATIENTS AND METHODS: Blood samples from Stage II-III patients enrolled on a prospective, randomized trial of neodjuvant, sequential doxorubicin and paclitaxel vs paclitaxel and doxorubicin were taken pre, during and post-chemotherapy and at 6 month intervals during follow-up. Peripheral blood mononuclear cells were isolated and enriched for epithelial cells by immunomagnetic selection (DynabeadsTM). The presence of CK19 gene mRNA was determined by RT-PCR using the LCx probe system (Abbott Laboratories).

RESULTS: CK19 mRNA was undetectable in 29/30 healthy controls. Of 27 patients who have completed neoadjuvant chemotherapy to date, 10/27 (37%) were positive for CK19 expression at baseline. CK-19 positivity was associated with T3 vs T2 lesions (p=0.008). After the first single agent, 7/27 (30%) patients were positive for CK19. Three of 27 (11%) remained positive after the second single agent, prior to surgery. No correlation with clinical response in the breast was seen. Pathologic CR was too infrequent to make meaningful comparisons. Of the three patients positive for CK19 after neoadjuvant therapy, one had a concomitant rise in tumor markers (CEA, CA 27.29) and a clinical relapse 6 months after completing therapy. At 6 months follow-up, another 3 patients have developed detectable CTCs in the peripheral blood. One of these three had a concomitant rise in both CEA and CA27.29, without clinical relapse as yet.

CONCLUSIONS: Results of this study suggest that CTCs are detectable by RT-PCR for CK19 in a significant proportion of Stage II-III breast cancer patients and correlate with larger tumor size. CTCs decline with neoadjuvant chemotherapy and may herald early relapse. Further follow-up will be presented for patients on this trial.

215 Circulating epithelial cells are elevated in women with a suspicious breast mass.

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Background: Previous studies investigating circulating epithelial cells (CEC) focused on advanced disease. The objective of this prospective study was to determine if CEC exist in healthy women, women with benign and malignant breast disease, and pre/post surgery in women with breast cancer.

Materials and Methods: CEC were determined in 100 healthy women after a negative routine screening mammogram and 186 women with a suspicious radiographic and/or palpable mass prior to FNA or biopsy. CEC were enumerated in 30mL of peripheral blood prepared and analyzed using Immunicon's *CellSearch™* system. Assay accuracy and reproducibility were established using 30mL of normal blood (n=30) spiked with ~10 tumor cells. CEC were defined as EpCAM (+), nucleated, cytokeratin (+), CD45 (-) cells. Patients with cancer were re-sampled prior to and after curative surgery when possible. CEC counts were compared to pathology and analyzed for trends.

Results: Assay accuracy = 74% (average spiked tumor cell recovery); Reproducibility = 64 - 85% (95% CI).

Category		Summary of CEC Counts in All Patients		Summary of CEC Counts in Patients with ≥2 Cells*		
Diagnosis	**N (%)**	**Mean ± SD**	**Range**	**# Positive (%)**	**Mean ± SD**	**Range**
Normal Mammogram	95**	0.2 ± 0.5*	0 → 2	2 (2%)	2.0 ± 0.0	2 → 2
Suspicious Mass (Pre Biopsy Sample)	186 (100%)	1.4 ± 2.8	0 → 22	47 (25%)	4.8 ± 3.8	2 → 22
—> Benign	145 (78%)	1.3 ± 2.9	0 → 22	36 (25%)	4.9 ± 4.1	2 → 22
—> DCIS / LCIS	10 (5%)	0.8 ± 1.0	0 → 3	2 (20%)	2.5 ± 0.7	2 → 3
—> Invasive Cancer	31 (17%)	1.6 ± 2.6	0 → 12	9 (29%)	4.6 ± 3.3	2 → 12
Pre Surgery Sample	35	4.9 ± 18.4	0 → 108	11 (31%)	15.1 ± 31.1	2 → 108
Post Surgery Sample	35	1.2 ± 1.8	0 → 8	11 (31%)	3.4 ± 1.7	2 → 8

* Upper limit of normal = 1.7 CEC/30mL (mean ± 3 SD). **Five were excluded as outliers (i.e. ≥ mean ± 3 SD).

Discussion: In 25% of women with a suspicious breast mass, CEC/30mL were elevated compared to normal healthy women (p=0.0001). No significant differences in CEC counts were observed between the benigns and cancers. After curative surgery, 31% of patients still had elevated CEC. Long-term follow-up, genotyping and phenotyping of CEC are required to determine their clinical impact.

216 Cell filtration: a simple method to isolate putative cancer cells from the blood of patients with metastatic breast cancer.

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Epithelial cells may be detected in the peripheral blood of many patients with breast cancer.In some cases these cells have been shown to have the characteristics of malignant cells.Identification and quantification of such cells might enable the early detection of relapse and the monitoring of response to treatment. Additionally, analysis of biomarkers expressed by the cells may allow more rational therapeutics and be a valuable tool in drug development.We have developed a simple inexpensive method for the detection and characterisation of these cells.Blood is filtered through polycarbonate membranes that have been punctured with calibrated microscopic cylindrical pores 8µm in diameter.Red and white blood cells pass through the filter while the larger malignant cells are trapped.We have found a level of epithelial cell enrichment similar to that by immunomagnetic separation. Cells on the membrane are fixed in ethanol,stained with propidium iodide (a fluorescent nuclear stain) and anti-pan-cytokeratin-FITC (to identify epithelial cells.) The filters are then examined by Laser Scanning Cytometry,which allows enumeration and localisation of cells.The detected cells can then be further characterised either by using an additional antibody or by restaining the slide and relocalising the cells (whose x/y corodinates have been stored.)In recovery experiments using blood samples spiked with known numbers of T47D cells we have demonstrated excellent linear recovery of cells down to single numbers of cells/ml.We have also analysed blood from 20 patients with metastatic breast cancer and from 6 normal control subjects.Nine or 18ml of blood were taken from each patient, filtered and stained as descibed above. Following scanning, cells provisionally reported as positive were relocalised for morphological confirmation.Where there was difficulty in verification the filters were restained with H&E and the suspect cells relocalised. No epithelial cells were identified in the blood of normal subjects. Epithelial cells were identified in all 20 patients with metastatic breast cancer.The mean number of cells identified was 1.8/ml(range 0.22-5.67.) We are currently validating this method in patients with all stages of breast cancer. We are also developing protocols to analyse the phenotype of cells with respect biomarkers such as HER2,EGFR,ER and PgR. If validated in our ongoing studies this technique could become a valuable tool in the management of patients with breast cancer. The filtration step is simple and could be performed in a ward side-room; the fixed membranes could then be transported to a centralised laboratory for staining and scanning.

We acknowledge the help of the Breast Cancer Campaign in supporting this project

217 Quantitative real-time RT-PCR for the detection of circulating breast cancer cells: correlation with stage and treatment.

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OBJECTIVE: Real-time RT-PCR (qPCR) can detect small numbers of breast carcinoma cells in the peripheral blood via quantification of keratin-19 (K19) mRNA. After developing a K19 qPCR assay, we collected serial blood samples in breast cancer subjects to determine the correlation of circulating tumor cells (CTCs) with stage and treatment.

METHODS: RNA was isolated from Ficoll-purified peripheral blood mononuclear cells collected from 56 normal volunteers and 54 breast cancer subjects. Samples were enriched for epithelial cells using either BerEp4 antibody immunomagnetic beads (Dynal) or by depletion of hematopoietic cells using Rosette-Sep (StemCell Technologies) before performing qPCR using the ABI 7700 system (Applied Biosystems). Breast cancer subjects had repeat blood samples drawn every six months.

RESULTS: Blood samples from normal volunteers spiked with SKBR3 cells revealed that K19 mRNA was detectable by qPCR down to one SKBR3 cell per 10E-7 WBCs. Twenty-seven of the 52 adult female normal volunteers had detectable K19 mRNA levels, and those levels were used to determine a 95th percentile reference range for CTCs. Fourteen of 54 (26%) cancer patients had K19 mRNA levels consistent with CTCs. All Stage I patients tested negative, whereas 27% of Stage II-IV patients tested positive for CTCs. Follow-up clinical data is available for 39 of the patients over an average of 6.4 months. Follow-up blood samples are available on 16 of the 39 patients. All but one of the repeat K19 levels correlated with clinical response. Eleven of the 16 had measurable disease: 4 had clinically detectable metastases, and 7 had locally advanced disease. Seven of the 16 originally tested positive for CTCs. K19 levels for all 7 of these subjects fell to below reference range for CTCs while receiving chemotherapy. Once CTCs were cleared, none have reappeared during the short follow-up available thus far. Clinically, one of these subjects did have disease progression, however, only at known sites of metastasis. None of the subjects with measurable disease who did not have CTCs at baseline have had subsequent positive K19 levels, and all of them are either NED or have at least achieved partial response so far.

CONCLUSIONS: These results suggest that K19 qPCR is useful for detecting CTCs, and that CTCs are more commonly found with Stage II-IV disease. The presence of CTCs at baseline may predict for systemic therapy response, and CTCs appear to clear with the administration of chemotherapy, but it is not yet clear whether CTCs will reappear prior to disease progression at already established sites.

218 Topoisomerase II-α amplification as potential predictive marker of complete response to anthracycline-based chemotherapy in locally advanced/metastatic breast cancer.

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Background: Most breast cancer pts receive CT during their disease evolution. Anthracycline (A)-based regimens are among the most active but also yield important side effects. Predictive markers (PM) of response to this CT are essential. HER-2 role is still under evaluation. Topoisomerase-IIα (topo-II) is the target of A and preliminary data suggest a promising role. **Methods**: After screening a population of 350 pts, two subgroups were identified: the study group (30 pts) composed of 13 complete responders (CR-a) and 17 true non-responders (TNR) (PD-a) to A-based CT, and the control group (26 pts) with 6 CR (CR-c) and 20 TNR (PD-c) to taxane-based CT. TNR were defined as progressive disease (PD) within the first three cycles. HER-2 and topo-II were evaluated by FISH in archival tumor samples (Vysis multi-color probe-positivity cut-off: ≥2 ratio for HER-2 and ≥1.5 for topo-II) and results were correlated with clinical response. **Results**: Both groups of CR and both groups of PD are well balanced for age, number and type of metastases. Pts with ≥2 previous lines of CT: 0/13 (CR-a), 3/17 (PD-a), 3/6 (CR-c), 9/20 (PD-c). Results are reported in the table. All cases HER-2-negative were also topo-II-negative. **Conclusions**: 1) Analysing the two "extreme scenarios" regarding response to CT, this study does not seem to support the usefulness of either HER-2 or topo-II as PM for CR to A. 2) Since it is a retrospective study with small number of pts, no definitive conclusions can be drawn, and a prospective study is ongoing. 3) Topo-II protein overexpression can occur without gene amplification, and it might be important for prediction of A efficacy. Evaluation of topo-II protein is ongoing.

	Anthracycline group (30 pts)		Taxane group (26 pts)	
	CR (13 pts)	PD (17 pts)	CR (6 pts)	PD (20 pts)
HER-2 amplified	5 pts (38%)	5 pts (29%)	3 pts (50%)	5 pts (25%)
Topo-II amplified	3 pts (23%)	1 pt (6%)	1 pt (17%)	2 pts (10%)
Topo-II non-amplified/deleted	2 pts (15%) / 0 pt	4 pts (24%) / 0 pt	2 pts (33%) / 0 pt	2 pts (10%) / 1 pt (5%)
HER-2 non amplified	8 pts (62%)	12 pts (71%)	3 pts (50%)	15 pts (75%)

219 Evaluation of topoisomerase II-alpha as a predictor of clinical and pathological response to neoadjuvant chemotherapy in operable breast cancer.

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Background: Topoisomerase II-alpha (topo II-alpha) is a key enzyme in DNA replication and a molecular target for anthracycline chemotherapy. We compared topo II-alpha expression with clinical and pathological response to primary anthracycline chemotherapy in operable breast cancer.

Materials and methods: 113 patients with T2-T4 N0 or N1 breast cancer (median age 48 yrs, range 26-78) received 6 cycles of chemotherapy (FEC n=92, AC n=8, MMM n=13) followed by definitive surgery. Response was assessed using both UICC clinical criteria and a simple pathological scoring system (CR = no residual invasive carcinoma; PR = residual tumour with evidence of chemotherapy-induced changes and a reduction in tumour cell : stroma ratio; NR = residual tumour with no chemotherapy effect). Immunohistochemical staining for topo II-alpha was performed on diagnostic core biopsies (n=113) and surgical specimens (n=89) using a monoclonal antibody (Harris et al). Tumours expressing nuclear staining in > 25% of cells were scored positive.

Results: 41% of biopsies and 35% of surgical specimens were topo II-alpha +ve. Topo II-alpha expression was significantly associated with Ki-67 (p=0.01) and ER (p=0.03) but not PgR or HER 2. 7 of 8 pathological complete responders were topo II-alpha +ve before chemotherapy (p=0.01). 71% of tumours showed no change in topo II-alpha staining after treatment; 14% displayed reduced expression (+ve to -ve); 14% demonstrated increased expression (-ve to +ve). Tumours with increased topo II-alpha staining after chemotherapy were less likely to respond by pathological criteria (8%) than those with unchanged or decreased expression (67% and 50% respectively, p=0.06).

Conclusion: Topo II-alpha expression fails to predict clinical response to neoadjuvant chemotherapy. There is a trend towards better response by pathological criteria in overexpressors. Tumours with increased expression after treatment have low pathological response rates.

Clinical (cRR) and pathological (pRR) response rates:

	All patients	Topo-II alpha +ve biopsy	Topo-II alpha -ve biopsy	p
cRR	78%	80%	76%	0.59
pRR	39%	49%	33%	0.12

220 Gene expression profiling of fine needle aspirations of breast cancer identifies genes associated with complete pathologic response to neoadjuvant taxol/FAC chemotherapy.

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The identification of molecular markers that predict response to neoadjuvant chemotherapy could lead to optimal treatment selection for individual patients with breast cancer. The goal of this ongoing prospective study is to identify expression profiles that may predict complete pathologic response (pCR) to preoperative FAC/Paclitaxel therapy. We obtained fine needle (FNA) biopsies of breast cancer before any systemic therapy, RNA was extracted from each sample and profiled without further amplification on cDNA microarrays containing 30,000 human transcripts. We successfully isolated RNA and performed profiling on 72% of all cases. The first 24 cases were used as discovery set to identify genes associated with pCR defined as complete disappearance of invasive cancer from the breast and lymph nodes. The next 11 cases were used to test the predictive power of our marker genes. Six of the 24 patients in the discovery set (25%) achieved pCR. To identify genes associated with pCR we calculated for each clone the signal-to-noise ratio values [absolute ($\mu pCR-\mu<pCR)/(\sigma pCR+\sigma<pCR)$], where μ and σ represent the mean and standard deviation of expression for each class pCR and < pCR (any residual invasive carcinoma). The top 150 clones ranked by signal-to-noise ratio were used for supervised clustering and correctly classified all the patients in the discovery set to either pCR or < pCR. Genes that were identified are involved in a variety of cellular functions including signal transduction, cytoskeletal organization and cell cycle regulation. Eleven additional cases have also been profiled and assigned a response category based on transcriptional profiling. These patients currently receive chemotherapy and pathologic response results will be presented during the meeting. In the future, these results may assist physicians to identify patients based on the transcriptional profile of their tumor who benefit most from FAC paclitaxel combination therapy.

221 Breast cancer gene expression profiles predict response to neoadjuvant taxotere.

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Background: cDNA array-based genetic expression profiles for primary breast cancers have great potential to define genetic heterogeneity and prioritize specific treatment strategies. Adjuvant chemotherapy improves surgical control of primary breast cancer and reduces the risk of recurrence. Adjuvant taxotere (docetaxel) therapy has one of the highest response rates, but *de novo* resistance or incomplete response to this therapy is frequent. We hypothesize that specific patterns of gene expression can predict which breast cancers will be susceptible or resistant to docetaxel therapy.

Materials and Methods: Clinical response was determined in 40 patients after 12 weeks of neoadjuvant taxotere therapy. Total RNA was extracted from pre-treatment core biopsies from the 24 patients representing the extremes of treatment success or failure. Double-stranded cDNA was synthesized using a chimeric oligo-dT/T7 RNA polymerase promoter oligonucleotide. Labeling and linear amplification was accomplished by *in vitro* transcription in the presence of biotinylated ribonucleotides. Affymetrix HG-U95Av2 chips were used to determine gene expression patterns of 12,000 genes. Expression levels were quantitated and analyzed with Affymetrix Microarray Suite and DNA Chip Analyzer software (dChip).

Results: After filtering on signal intensity to eliminate genes with uniformly low expression, we selected 747 genes. We employed unsupervised hierarchical clustering analysis, and these genes separated tumors into responder, partial responder, and progressive disease groups. Supervised analyses including differences in average expression ratios of expression, two sample t-tests and Wilcoxon rank sum tests define a subset of approximately 100 genes that predict likelihood of response to docetaxel therapy. Non-responder tumor profiles exhibit elevated microtubule components, responders appear to be under stress, and have elevated levels of microfilament, immune, inflammatory response, heat shock and mitochondrial genes. Semi-quantitative RT-PCR studies of differentially expressed genes confirm the hybridization-based data for the arrays. Immunohistochemical studies are underway to confirm observations at the protein level.

Discussion: The clinical heterogeneity of breast cancer remains the primary barrier to treatment success. cDNA array-based expression profiles appear capable of predicting response to single agent taxotere therapy. Identification of individual genes or array-based patterns of gene expression for use as a simple predictive test for docetaxel sensitivity would reduce unnecessary treatment, toxicity and cost to women with breast cancer.

222 Significance of mismatch repair-protein-expression in regard with chemotherapeutic response of sporadic invasive ductal carcinoma of the breast.

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Background: Mismatch repair (MMR) gene plays a key role in the correction of DNA damage, and the loss of MMR has been implicated in resistance to a variety of chemotherapeutic drugs. The purpose of this study was to assess whether the reduced expression of hMLH1 and/or hMSH2 affects the chemotherapeutic responsiveness in sporadic, invasive, ductal cancer of the breast.

Material and Methods: Immunohistochemical studies were performed on 71 histological specimens of breast cancer taken from the patients treated by curative operation and subsequent cyclophosphamide-methotrexate-5-fluorouracil (CMF) or cyclophosphamide-adriamycin-5-fluorouracil (CAF) chemotherapy for stage II or III primary breast cancer. PCR-SSCP and sequencing were carried out in 16 patients. A combined immunoreactivity score (hMLH1-IS and hMSH2-IS) was calculated by multiplying percentage staining grade by intensity score.

Results: Positive expression (>4) of hMLH1-IS and hMSH2-IS were 57.7% and 60.6%, respectively and complete losses of hMLH1 and hMSH2 were observed in 4.2%. Of patients with advanced cancer with lymph node metastasis, those having a low level hMLH1-IS showed significantly higher failure rate to CMF regimen than those having a high level hMLH1-IS (p=0.03). No significant difference was noted in chemotherapeutic response according to hMLH1 and hMSH2 expression in the CAF group. Both hMLH1-IS (p=0.03) and status of progesterone receptor (p=0.03) well correlated with CMF chemotherapy response in the breast cancer with lymph node metastasis.

Discussion: Our study supports that the lack of hMLH1 expression may play a role in drug resistance especially in the CMF group, and immunohistochemical assay for MMR protein could be used as a convenient tool for evaluating chemotherapeutic response in patients with breast cancer.

223 Impact of pretreatment hemoglobin levels on tumor response, microvessel count and Ki67 expression in breast cancer patients undergoing primary chemotherapy.

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We investigated the impact of hemoglobin (Hb) levels on clinical and biological features in breast cancer patients receiving primary chemotherapy. From 1990 to 1997, 2 consecutive phase II studies of primary chemotherapy were conducted in our Institution. These studies involved 157 breast cancer patients with operable or locally advanced disease (T2-4N0-1M0). The first 76 received the CMF regimen + tamoxifen, the remaining 81 were submitted to single agent epirubicin. Tumor specimens were obtained before chemotherapy by incision biopsy and at definitive surgery, respectively. Tumor shrinkage (>50%) was obtained in 113 out of 156 evaluable cases (72.4%). Pathological complete response was attained in 3 (2%) cases. The mean Hb levels (+SD) at baseline conditions was 13.5 g/dl (+1.2) in responding patients and 13.13 (+1.0) in non-responders (p=0.06). The corresponding figures of red blood cell count (RBC) were 4.56 (+0.39) and 4.35 (+0.36), respectively (p <0.01). A cut off of 13 g/dl was defined to discriminate between normal and low Hb levels. Patients with a starting condition of low Hb levels obtained a lower response rate than their counterpart [36/61 (59.0%) vs 77/95 (81.0%), p < 0.003]. All patients attaining complete pathological response had normal pretreatment Hb. Ki 67 immunostaining at baseline condition did not differ comparing patients with low [median 14.5 (range 1-50)] or normal Hb [16.0 (range 1-90)], but low baseline Hb status was predicitve of higher Ki67 expression in post operative BC specimens [median 9.5 (range 0-80) vs 5 (range 0-50), p<0.03]. Blood microvessel count (MVC) by CD34 immunostaining were available before and after treatment in 120 matched pair breast cancer samples. There was a low but significant inverse relationship between Hb levels and MVC either before (Spearman r - 0.23) and after treatment (Spearman r - 0.24) (p<0.05). To conclude, baseline Hb status significantly predicted treatment response in breast cancer patients undergoing primary chemotherapy. Our data also suggest a possible contribution of Hb levels in influencing the tumor-induced neoangiogenesis and the antiproliferative effect of the cytotoxic treatment.

224 Factors predicting the response to postoperative adjuvant therapy with tegafur-uracil (UFT) in women with breast cancer.

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Background: The reduction in the odds of recurrence has been reported to be 21% with tegafur-uracil (UFT), an orally available prodrug of 5-fluorouracil. Establishment of factors predicting the response to UFT can help to provide simple, effective treatment to patients. To examine predictive factors in breast cancer patients receiving UFT as postoperative adjuvant chemotherapy, we retrospectively studied the relation between tumor expression of thymidylate synthase (TS), the target enzyme of 5-FU, and suppression of recurrence by UFT.

Material and Methods: We analyzed premenopausal patients with axillary lymph node metastasis from among patients registered in randomized controlled trials (the third ACETBC study), evaluating UFT as postoperative adjuvant chemotherapy in breast cancer. Immunohistochemical studies were done with the use of recombinant human TS polyclonal antibody. Three pathologists independently evaluated the results.

Results: Specimens were retrieved from 204 patients. Specimens from 192 patients (UFT group,n=95; non-UFT group,n=97) could be evaluated. A total of 55% of the specimens (105/192) were TS positive. Within the TS-positive group, the 5-year recurrence-free survival rate was significantly better in the UFT group than in the non-UFT group (log-rank test p=0.0766, g. wilcoxon test p=0.0442). In the TS-negative group, there was no appreciable effect of UFT.

Discussion: Our results suggest that TS may be a useful factor for predicting the response to UFT. Studies of thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) are scheduled to be performed.

225 Measurement of intratumoral choline levels by magnetic resonance imaging and spectroscopy as a marker of response to chemotherapy.

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Response to pre-operative chemotherapy for early stage breast cancer patients is a powerful predictor of outcome (Fisher et al. J Clin Oncol 16:2672,1998). Immediate detection of chemotherapy response would be beneficial, as ineffective treatment regimens could be immediately identified and discontinued. In this study, we used magnetic resonance imaging (MRI) and spectroscopy (MRS) to measure choline levels in an animal model of drug resistance and in patients undergoing neoadjuvant therapy. Choline has been identified as a metabolic marker for breast cancer. To determine if changes in choline levels could be detected in xenograft tumors and predict response to chemotherapy, we implanted parental MCF-7 and doxorubicin resistant MCF-7 (MCF-7 A/F) cells in athymic mice. MCF-7 A/F were selected for doxorubicin resistance (De Larco et al. Cancer Res 61:2857, 2001) and fail to undergo doxorubicin-induced apoptosis in vitro. High choline levels were detected in both parental MCF-7 and MCF-7 A/F by MRS at 4.7 Tesla (T). When animals were treated with 8mg/kg doxorubicin and re-examined 24 hours later, only the parental xenograft tumors had reduced choline levels suggesting that choline response may identify chemotherapeutic sensitivity. To determine if similar findings can be measured in human breast cancer, we are performing a clinical study to perform MRI/MRS at 4T in women undergoing neoadjuvant therapy for locally advanced breast cancer. In this study, patients are imaged prior to receiving AC chemotherapy and 24 hours later. Additional scans are obtained prior to surgical resection. One patient has been particularly instructive as she had multiple lesions in the breast. A large DCIS had elevated choline levels unaffected by AC. Two other invasive lesions had elevated choline levels that were diminished after the first cycle or second cycle of AC. One of these lesions achieved a pathological complete response after 4 cycles of AC. We conclude that choline levels can be measured by high magnetic field MRS in primary breast cancer. Furthermore, suppression of choline levels after doxorubicin-based chemotherapy is an excellent predictor of tumor response.

226 A phase II trial of pemetrexed in previously untreated breast cancer.

Gomez H, Hanauske A-R, Santillana S, Vallejos C, Paoletti P, Robb C, Lee D, Penn K, Hockett R, Chen V, Niyikiza C. Instituto de Enfermedades Neoplasicas, Lima, Peru; St. Georg Hospital, Hamburg, Germany; Eli Lilly and Company, Indianapolis, IN

Pemetrexed (ALIMTA) is a novel antifolate with demonstrated activity in locally advanced and metastatic breast cancer. Molecular targets include folate dependent enzymes involved in both pyrimidine and purine neosynthesis. This Phase II study was designed to investigate whether a correlation exists between expression of targeted molecular markers and clinical response using pemetrexed given as a single agent in the neoadjuvant setting to patients (pts) with advanced disease. Tumor biopsies were taken prior to drug exposure, 24 hrs after the initial dose, and following [a maximum of] three cycles of pemetrexed. Pemetrexed was dosed at 500 mg/m2 IV over 10 minutes every 21 days. Low-dose folic acid, vitamin B12, and dexamethasone were given to all pts. Sixty-one pts were enrolled and treated on the trial, all of which have clinical data available. Nineteen pts achieved partial response, for an overall response rate of 31%. Tissue analysis is ongoing. Tumor tissues are being evaluated for mRNA expression of thymidylate synthase (TS), dihydrofolate reductase (DHFR), glycinamide ribonucleotide formyltransferase (GARFT), p53, and erbB2 by RT-PCR; for immunohistochemical staining (IHC) of TS, DHFR, and GARFT; for p53 mutations with single-stranded conformation polymorphism (SSCP); and for c-erbB2 expression with fluorescent in-situ hybridization (FISH). Early results indicate that ↓TS expression at baseline may correlate with clinical response. Expression levels in tissue samples from subsequent biopsies suggest that over time increased levels of TS are associated with resistance to therapy with pemetrexed. Analyses of biopsies obtained at baseline and subsequent to drug exposure are anticipated to provide information on the modulation at both the gene and functional levels of the targeted molecular markers. Transcript profiling is planned to expand on these observations, and will likely yield further correlations. The complete correlative analysis of the relevant biomarkers and their relationship to response is in progress and will be reported.

227 Anthracycline based chemotherapy in locally advanced breast cancer. Her 2/neu expression as a predictive factor for resistance.

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INTRODUCTION: Breast cancer is the second most frequent cancer in Mexico, 30 to 50% are locally advanced , therefore neoadjuvant chemotherapy is required, usually with antracyclics. The over expression of Her2 neu implies a bad prognosis but good response to antracyclics. The objective of our study was to evaluate the expression oh her2 neu clinical and histological characteristics , to identify response predictive factors to antracyclics in locally advanced breast cancer, to adequate chemotherapy schemes.

MATERIAL AND METHODS: Clinical records from patients treated with neoadjuvant chemotherapy with antracyclics at the institution for clinical stage III breast cancer between 1992-97were reviewed. Demographic, clinical ,treatment and response data were collected. Slides and blocks were reviewed. Her 2neu expression was determined with herceptest and ploidy with cytophotometry.

RESULTS: Patients were divided in two groups ::A with 56 cases responding to chemotherapy and B 20 non responders.. Mean age was 50.1 and 47 for groups A and B respectively with significance (OR 7.02 p=0.004). Were premenopausal 43% and 70% from groups A and B respectively (OR 3.1 p=0.04) Clinical tumour mean size was 5cm in responders and 8 cm for non responders (OR 4 p=0.02). Clinical stage III-B were 16% and 70% for groups A and B (OR12.2 p=0.000). All were high grade tumours, aneuploid 39% of responders and 18.7% for non responders. Her 2 neu was over expressed in 64.2% and 50% for groups A and B (OR 3.6 p=0.06). On multivariate analysis significance was conserved only for age, stage and size.

CONCLUSIONS: With our results, we can observe that young patients, clinical stage III-B and tumours > 5 cm had lower response rate. In the responders a higher percentage of her 2 neu over expression was observed, and probably without significance due to sample size. It would be important to study a bigger group to decide if in our population can be a response predictive factor the over expression of her 2 neu and include the Herceptest in the routine tests.

228 The feasibility of Her-2 testing in metastatic breast cancer specimens obtained by needle biopsy.

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Background: There is a growing interest in the predictive value of the HER-2 overexpression in stratifying breast cancer patients for Herceptin therapy. Since only 43% of newly diagnosed breast cancers will metastasize, it may be cost-effective to limit HER-2 testing to metastatic lesions before Herceptin therapy. Fine needle aspiration biopsy (FNAB) or core needle biopsy (CNB) are excellent in sampling superficial or deep-seated metastatic lesions. This study was designed to assess the pattern of HER-2 overexpression of metastatic breast cancer and its primary lesion; and the reliability of HER-2 testing in specimens obtained by either FNAB or CNB compared to the surgical resection specimen.

Materials and Methods: There were 63 primary breast cancers with corresponding metastatic lesions. Most tumors metastasized to lymph nodes and 17 cases metastasized to other sites. There were 94 needle biopsy specimens (40 FNAs and 54 CNBs). Twenty FNABs and 26 CNBs had corresponding surgical specimens. HercepTest™ (DAKO Corp., Carpinteria, CA) was performed on these specimens following the protocol and scoring guidelines recommended by the manufacturer.

Results: HER-2 overexpression was observed in 37% (23/63) cases. The pattern and intensity of HER-2 overexpression were found to be nearly identical in primary and metastatic lesions. HER-2 positive primary tumors remained positive also in the corresponding metastatic lesions (except in one case). HER-2 negative primary tumors had HER-2 negative metastases. Intratumoral heterogeneity was noted. The concordance between FNAB/CNB and surgical specimens was statistically significant ($p<0.001$). The disconcordant cases were: 1+ positive in CNB compared to 2+ in the surgical specimen; negative in FNAB compared to 2+ in the surgical specimen.

Conclusions: It is feasible to assess HER-2 overexpression of metastatic breast cancer specimens obtained by needle biopsy. HER-2 testing in FNAB or CNB is a reliable alternative in selecting patients for Herceptin therapy.

229 Evaluation of Ki67 proliferation index before and during neoadjuvant chemotherapy for primary breast cancer.

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Background: The cell proliferation marker Ki67 may be of value in determining response to treatment for breast cancer. This study measured the Ki67 cell proliferation index on histological material before, during and after primary anthracycline chemotherapy for operable breast cancer in order to assess the utility of Ki67 as a clinical tool for predicting response to chemotherapy.
Materials and methods: 27 patients (median age 53, range 29 - 65) underwent diagnostic core biopsy and six cycles of neoadjuvant FEC chemotherapy followed by definitive surgery. Core biopsy was repeated on day 21 of treatment, immediately before the second cycle of chemotherapy. Immunohistochemical staining for Ki67 was performed on diagnostic and day 21 biopsies and surgical specimens. A proliferation index (ratio of stained : non-stained cells in 10 high power fields) was calculated by manual cell counting.
Results: The median (range) Ki67 index (%) at diagnosis, day 21 and surgery was 27.9 (4.1 - 43.9), 17.3 (4.1 - 44.8) and 21.7 (2.4 - 50.4) respectively. There was no significant difference in baseline Ki67 between responders and non-responders when assessed by clinical, radiological or pathological criteria. Clinical responders were more likely to exhibit a fall in Ki67 index after one cycle of chemotherapy (median - 50.6, range - 73.0 to 93.3) than non-responders (median - 5.3, range - 43.4 to 57.7), p=0.036. In responding patients, the reduction in Ki67 index after one cycle of treatment was often followed by a rebound increase in cell proliferation by the time of surgery (responders 26.8 (range 2.4 to 48.0); non-responders 18.9 (6.8 to 50.4)).
Conclusions: Clinical response to chemotherapy is often preceded by a fall in Ki67 index after one cycle of treatment. The observed changes in proliferation during treatment may have implications in determining the optimum duration of neoadjuvant chemotherapy prior to surgery for operable primary breast cancer.

230 Analysis of disease-free and overall survival according to immunohistochemically determined ER, PR and EGFR levels in a large series of primary breast cancers.

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Background: Estrogen receptor (ER) positivity is predictive for the effectiveness of endocrine therapy in breast cancer (BC), given either as adjuvant treatment or for advanced disease. It has not been established however what the effects of different levels of ER or progesterone receptor (PR) are or whether there is a cut-off level among positive values that predicts endocrine treatment efficacy. ER and PR are most commonly measured by immunohistochemistry (IHC), using specific antibodies to label the receptors in frozen or paraffin embedded tumor specimens. We have updated our analysis of a database of 946 patients in whose tumours ER and PR was detemined prospectively between 1987-1995.
Methods: ER, PR and EGFR were detemined on frozen sections by IHC and positivity was expressed as a percentage of positive cells per total cells counted for ER and PR. We have shown that results obtained by this method correlate well with those obtained by IHC on paraffin embedded tumor specimens. EGFR was classified as positive with any degree of positive staining. Kaplan-Meier survival curves were constructed for disease-free survival (DFS) and overall survival (OS) and compared using the Log Rank method
Results: Median follow up was 5.3 years. There were 232 BC relapses and 187 BC deaths . In patients who had received adjuvant endocrine therapy, ER values in the range 21- 40% were associated with significantly better DFS and OS than those with negative ER (p =0.019 and 0.017 respectively). For ER 1-20%, OS and DFS were intermediate between ER 0% and 21-40 but were not significantly different to ER 0%. PR 1-20% had significantly better DFI and disease specific survival than PR 0%. In ER positive patients who received adjuvant tamoxifen, EGFR+ patients had a significantly worse DFI than EGFR negative patients.
Discussion: We conclude that ER levels > 20% are predictive for endocrine responsiveness of primary breast cancers. A smaller, but clinically significant benefit from endocrine therapy in patients with ER 1-20% cannot be excluded. EGFR and PR may provide useful information in further delineating the benefits of endocrine therapy.

231 Changes in tumor proliferation following neoadjuvant tamoxifen treatment may predate response and predict for relapse.

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Background: Tamoxifen's utility may be limited by primary and acquired resistance. Neoadjuvant treatment coupled with sequential biopsies allows effects on tumor proliferation to be monitored and related to tumor response/ resistance and patient outcome.
Materials and Methods: Two separate cohorts of postmenopausal women with ER-rich breast cancer were studied - 1) 51 patients who had a pre-treatment biopsy and definitive surgery after 3 months treatment and had at least 5 years subsequent follow-up and 2) 49 women who had tumor cores taken before and after 14 days and 3 months of treatment. Tumor volume was measured at monthly intervals and response classified as >25% reduction in volume. Proliferation was measured immunohistochemically by KiS1 staining (study 1) or Ki67 (study 2).
Results: *Study 1* - 37 of 51 (73%) patients responded to neoadjuvant tamoxifen, of these 23 (62%) had decreased tumor staining for KiS1 at 3 months. In contrast only 5 of 14 (36%) of non-responders had decreased staining. After 5 years follow-up 16 (31%) patients had relapsed, 9 (24%) in the responding group and 7 (50%) of non-responders. The relapse rate in the responding tumors which did not show a decrease in KiS1 (7/14) was identical to the non-responding group. *Study 2* - 38 of 49 (78%) patients responded. In responding tumors, 28 (74%) had decreased Ki67 staining by 14 days; but in 6 of these staining rose at least to pre-treatment values by 3 months. Conversely staining decreased at 3 months in 3 of the tumors originally having no change at 14 days. In contrast, only 3 (27%) non-responding tumors had decreased Ki67 staining at 14 days and the status did not change between 14 days and 3 months in any of the tumors.
Discussion: Response to neoadjuvant tamoxifen translates into longer-term clinical benefits. Changes in proliferation predate response in the majority of tumors. However a paradoxical lack of change in proliferation in clinically responding tumors is a phenotype which has the same poor 5 year relapse rate as non-responding tumors; this may represent early evidence of resistance to tamoxifen.

232 ErbB-2 amplification, ErbB-1 expression and tamoxifen response in ER-positive metastatic breast cancer; a SWOG study.

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From preclinical data expression of the ErbB family receptors may be involved in endocrine resistance. Clinical studies have produced varying results. Thus this study investigated the predictive value of amplification of ErbB-2 alone or in relation with ErbB-1 expression in women with ER-positive metastatic breast cancer treated with tamoxifen. The original trial began in 1982. ErbB-2 gene amplification and ErbB-1 expression were determined on 136 and 204 patients respectively. ErbB-1 was evaluated by immunohistochemistry. Fluorescence in situ hybridization (FISH) assay was used to detect ErbB-2 amplification. 23% of 136 (32/136) tumors showed ErbB-2 gene amplification and 10.7% (22/204) tumors overexpressed ERB-1. ErbB-2 amplification was correlated with lower ER (p=.02), higher ErbB-1 expression (p=.004) and ErbB-2 overexpression (p <.00001). Response (CR+PR+SD>6 months) was 56% for ErbB-2 unamplified vs. 47% for ErbB-2 amplified (p=.38), and 58% for ErbB-1 negative vs. 36% for ErbB-1 positive (p=.05). If both were negative, response rate was 57% vs. 43% if either were positive (p=.15). Time to treatment failure (TTF) was 7 months for unamplified ErbB-2 and 5 months (p=.007) for amplified ErbB-2 patients, and there was a trend towards a better overall survival (OS) in unamplified ErbB-2 patients (median 31 vs. 25 months respectively, p=.07). For high ErbB-1, TTF was 4 vs 8 months (p=.08) and median survival was 24 vs. 31 months (p=.41). Combining ErbB-1 expression and ErbB-2 gene status, patients with both negative ErbB-1 expression and unamplified ErbB-2 had longer TTF (p=.001) and OS (p=.03) vs. either positive. In a Cox model including for four independent variables (ER, PgR, disease-free interval, menopausal status) associated with TTF and OS in a previous study, SWOG 8228, ErbB2 had no independent value but ErbB1 was significant for TTF (p=.001). **Conclusion** In this group of ER-positive cancers, those with ErbB-2 amplification had lower ER levels but were only modestly less responsive to tamoxifen, with more than a third of patients continuing to respond. These data support the concept of the molecular heterogeneity of clinical breast cancer and suggest that molecular events other than these involving the ErbB family are important in determining the endocrine resistant phenotype.

233 HER2 as predictive marker of resistance to endocrine treatment (ET) for advanced breast cancer (ABC): a metanalysis of published studies.

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Backgroud: Experimental data suggest a complex cross-talk between the HER2 and the estrogen receptor (ER) and it has been hypothesized that HER2+ tumors may be unresponsive to ET. Clinical data, however, have been conflicting. We have previosly reported preliminary results of a metanalysis on the interaction between the response to ET and the overexpression of HER2 in ABC. Here we report an update of the metanalysis based on 12 studies and 2371 pts.

Methods: Studies have been identified by searching the Medline, Embase and ASCO abstracts databases. Selection criteria were: a) ABC pts treated with ET c)Responses assessed according to HER2 status. For each study the Relative Risk (RR) of progression for HER2+ over HER2- patients with 95% confidence interval (95%CI) was calculated as an estimate of the predictive effect of HER2. An overall estimate of the RR was computed by the Mantell-Haenszel method.

Results: the overall RR was 1.41 (95%CI=1.32-1.51; P<0.00001; test for heterogeneity: p=0.360), showing a highly significant correlation between HER-2 overexpression and TF. To avoid the potential confounding effect of the small number of ER-negative patients included in the global analysis, a second metanalysis was performed using data of patients who were either ER-positive, ER-unknown or ER-negative/PgR-positive. The overall estimate in this subgroup of patients (n=1,929) shows an even higher correlation between HER-2 overexpression and TF (overall RR=1.44; 95%CI: 1.34-1.56; p<0.00001; test for heterogeneity: p=0.260). In one study (Lipton 2002) in which pts had been randomized to Tamoxifen or Letrozole, there was evidence of resistance to therapy for HER2+ tumors in both arms, with RR=1.34 (95%CI: 1.12-1.60) for tamoxifen-treated versus RR=1.54 (95%CI: 1.24-1.88) for letrozole-treated pts.

Conclusions: the results of our metanalysis indicate that MBCs overexpressing HER2 are less responsive to ET. This effects holds in the subgroup of patients with positive or unknown steroid receptors. There is no evidence that Letrozole may overcome such a resistance in MBC.

234 Effect of tamoxifen on the expression of cyclin D1 in breast cancer and correlation with tumour response.

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Background: The role of biological mediators and early predictors of response to tamoxifen is crucial for optimising treatment.

Materials and Methods: The effect of tamoxifen on the expression of cyclin D1 was studied in 49 postmenopausal ER positive breast cancer patients receiving 20 mg of neo-adjuvant tamoxifen daily for 3 months. Immunohistochemistry for cyclin D1 was performed on sequential tumour biopsies obtained at diagnosis, following 10-14 days and 3 months of tamoxifen treatment. Results were expressed as cyclin D1 scores representing the intensity of nuclear staining (0=no staining, 1=mild staining, 2=moderate staining, 3=strong staining). Tumour response was defined as 25% reduction in tumour volume on ultrasound after 3 months of treatment. Analyses were performed to see if there were any changes in cyclin D1 expression over time and whether the changes could be used to predict response to tamoxifen.

Results:

	Median cyclin D1 scores and % with a score =2					
	Day 0	1st Biopsy	10-14 days	2d biopsy	3 months	3rd biopsy
All tumours (49 patients)	2.0	64%	2.0	59%*	1.5	50%**
Responders (37 patients)	2.0	66%	2.0	64%	1.0	48%**
Non-responders (12 patients)	2.0	58%	1.5	50%	2.0	54%

*p=<0.05; **p=<0.01

Conclusions: Although there is evidence that tamoxifen produced a decrease in cyclin D1 expression after 10-14 days and 3 months of treatment, there was no evidence that the changes in cyclin D1 could differentiate responding from non-responding tumours.

235 Concordance between local labs and a central lab using FISH and IHC for HER2 testing.

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Background: Herceptin has a significant survival benefit for HER2-positive MBC patients. It is essential that patients who may benefit from Herceptin be appropriately identified. Reports indicate a high incidence of false-positive and false-negative results when determining HER2 status by IHC and suggest that IHC obtained at local labs may not be as reproducible as those from central labs.

Patients and Methods: To evaluate the benefit of first-line Herceptin + a taxane in patients with HER2+ MBC prospectively selected by FISH, a phase IV multicenter study is being conducted throughout the US. Patients are eligible based on local or central HER2 testing (3+ by IHC or FISH+); local results are confirmed at a central lab (LabCorp). Samples with a HER2:CEP17 signal ratio ≥2 are considered HER2 gene amplified (Vysis PathVysion). IHC results are scored as 0, 1+, 2+, or 3+ (DAKO HercepTest).

Results: 706 pts have been screened, and 165 (23%) were deemed eligible based on a HER2 result of 3+ by IHC (n=146) or FISH+ (n=28) at their local lab. The majority of local tests were IHC; only 59 pts (8%) were screened locally by FISH. Of the 146 pts who were 3+ locally, 106 (73%) were also 3+ at LabCorp. Of the 268 pts who were <3+ locally, 39 (15%) were 3+ at LabCorp, and thus eligible for the study. 53 pts had both central and local FISH testing. Of the 28 pts who were FISH+ locally, 23 (82%) were confirmed centrally. 1 of 25 pts who were FISH- locally was FISH+ centrally. 40 of 251 (16%) pts screened by IHC and <3+ were FISH+ and thus among those who may benefit from Herceptin therapy.

Conclusions: These results are consistent with reports of inaccuracies in the determination of HER2 status by IHC and suggest that IHC performed at experienced labs demonstrating proficiency may yield more accurate results than IHC performed at local labs. In this study, local IHC failed to identify 15% of 3+ patients that were eligible for enrollment. FISH testing identified another 16% of patients that were not identified by either central or local IHC. Based on these results, in patients who have IHC scores <3+, consideration should be given to retesting with FISH. Additional data are needed to determine reproducibility of FISH. Preliminary data suggest that there may be higher reproducibility with FISH than IHC.

236 Correlation of HER-2/neu immunohistochemistry with fluorescence in situ hybridization in 3,568 breast cancers.

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Background: Breast cancer patients whose tumors express HER2/neu may benefit from targeted therapy by Herceptin®. Treatment eligibility has been determined by IHC, but FISH is now providing additional information. The lack of complete correlation between methods has created controversy for patient decisions. Testing variability between labs is well documented and has added to the confusion.

Materials and Methods: Since 1999, our laboratories have performed HER2 FISH on 13,828 breast cancers, of which 3,568 included IHC as well. Testing was standardized and validated, and training protocols implemented between two sites. IHC (DAKO HercepTest) and FISH (Vysis PathVysion) were performed following recommended procedures. Both medical technologists and pathologists completed standardized training to assure consistent results.

Results: The combined results from our LA and NY laboratories for consecutive breast cancer cases in which both HER2 IHC and FISH tests were ordered are shown in the table below:

IHC	FISH Positive	FISH Negative	Total	% Positive
0/1+	58	1,296	1,354	4.3
2+	380	1,393	1,773	21.4
3+	400	41	441	90.7
Total	838	2,730	3,568	23.5

Discussion: In a large cohort of breast cancer patients, our statistics are consistent with published results for IHC and FISH. IHC 2+ tumors are FISH positive in 21% of cases. Less than 5% of IHC 0/1+ specimens are positive for FISH and less than 10% of IHC 3+ cases are negative for FISH. The role of standardization and training has been critical.

237 A comparison of different methods for quantitative analysis of HER-2/*neu* status in invasive breast carcinoma by fluorescence in situ hybridization and immunohistochemistry by manual scoring and MDS™ image analysis.

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Background: Scoring of HER-2/*neu* immunohistochemistry (IHC) results in breast cancer is controversial and subject to inter-observer variability. Recent advances in automated image analysis technology offer the potential for increasing objectivity of the HER-2/*neu* scoring process. The aim of this study was to assess the accuracy of the Applied Imaging MDS™ image analysis system relative to standard IHC and FISH scored manually by pathologists.

Design: The HER-2/*neu* status of 56 cases of infiltrating breast carcinoma was assessed by three modalities: standard IHC, IHC with image analysis using the Applied Imaging system, and fluorescence in situ hybridization (FISH) of the HER-2/*neu* gene. The DAKO A0485 anti-HER-2/*neu* antibody was used for IHC; each case was scored by one of three pathologists and then independently analyzed by the MDS™ system. FISH was perfomed using the PathVysion™ kit (Vysis).

Results: In 32 cases originally graded as 0 or 1+ by pathologists, pathologists agreed with all of the values generated by the instrument. One case was amplified by FISH and received a score of 2+ by the instrument. Upon review, the pathologists concurred with the instrument score. In 14 cases graded as 2+ by pathologists, there was disagreement on 1 case that was non-amplified by FISH and manually scored as 2+. The instrument assigned a value of 3+ for this case. Of note, only one of the 2+ cases showed amplification by FISH. This same case received a value of 3.0 from the instrument, and upon review the pathologists agreed with the MDS™ score. In the 10 cases graded as 3+ by pathologists, 9 were amplified by FISH. The single case without FISH amplification received a value of 1+ by the instrument. The correlation coefficient was 0.92.

Conclusions: There was 100% concordance between pathologists and the MDS™ image analysis system in cases scored by pathologist as 0 or 1+, with an overall agreement of 93%. These results indicate that quantitative image analysis may improve manual subjectivity and interobserver variability in the interpretation of HER-2/*neu* expression, while maintaining accuracy of results.

238 Comparison of HER-2/neu status determined by fluorescence in situ hybridization (FISH) in the BCIRG central laboratories with HER-2/neu status determined by immunohistochemistry or FISH in outside laboratories.

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Background: HER-2/neu gene amplification and overexpression is found in approximately 20-30% of breast cancers. Women whose breast cancers show HER-2/neu gene amplification or overexpression have a poorer overall survival and are eligible for Herceptin (trastuzumab) immunotherapy. HER-2/neu status has become important in both clinical decision-making and in selection of women with breast cancer for entry to clinical trials. However, there is considerable disagreement regarding the accuracy and reproducibility of assay techniques used to assess HER-2/neu status. We evaluated the HER-2/neu status by fluorescence in situ hybridization (FISH) and compared these FISH assay results with the HER-2/neu status determined in other laboratories by either immunohistochemistry (IHC) or FISH.

Methods: FISH for HER-2/neu gene amplification was performed on paraffin-embedded breast cancer specimens submitted to either of two BCIRG Central Laboratories. The HER-2/neu gene amplification status determined by FISH in the BCIRG Central Laboratories for the first 2,600 breast specimens was compared with the HER-2/neu status determined in outside laboratories. Fifty-seven cases were excluded from this study for a variety of reasons.

Results: FISH showed high interobserver and interlaboratory agreement in the BCIRG Central Laboratories (kappa statistic: 97.1% and 97.1%). FISH was successful in 2,502 of the 2,543 (98.4%) cases included in this study. HER-2/neu gene amplification was observed in 655 of 2,502 (26.2%) breast cancers. Outside laboratories had assessed HER-2/neu status by IHC in 1,608 cases and by FISH in 121 cases. By IHC 656 (40.7%) showed overexpression (2+/3+). Overall the HER-2/neu status determined by IHC showed a 79% agreement rate with FISH performed by the BCIRG Central Laboratories; the DAKO Herceptest showed a 78% agreement rate and the Ventana Pathway assay showed a 79% agreement rate. The HER-2/neu status determined by unspecified FISH assay methods at outside laboratories showed a 92% agreement rate with FISH performed at the BCIRG Central Laboratories.

Conclusions: The findings presented here demonstrate that IHC assay methods used in outside referral laboratories have a relatively high rate of false-negative and false-positive results compared to FISH performed at centralized BCIRG reference laboratories. FISH performed at outside laboratories, on the other hand, showed a lower rate of both false-positive and false-negative results relative to FISH performed at centralized BCIRG reference laboratories.

239 Validating HER-2/neu assessment by immunohistochemistry.

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Background: The assessment of HER-2 by immunosthistochemistry (IHC) has been controversial. Studies utilizing enzyme immunoassay and radioimmuno labeling have shown that the level of HER-2 protein expression in breast carcinoma is bimodal. The bimodal distribution can be used to optimize IHC scoring as well as validate a specific IHC assay.

Material and Methods: 232 consecutive cases of invasive breast carcinoma were analyzed to validate the HercepTest™ Kit (Dako) and then to optimize the scoring system with the aid of image analysis. Optimization was performed by generating ROC curves using gene amplification as the true positive result. Gene amplification was assessed by FISH (PathVysion™, Vysis). The optimized scoring system, as predicted by the ROC plots, was then assessed in an additional 334 consecutive cases.

Results: All patients were female and ranged in age from 30-82 years, (mean 57). 16.4% of the original 232 cases were amplified by FISH. Fractional scoring performed by ACIS confirmed a bimodal distribution of HER-2 expression. ROC analysis suggested that an ACIS score exceeding 2.4, averaged from at least 6 distinct fields, was the optimal cutoff to predict gene amplification and that a maximum ACIS score of less than 1.9 was the optimal cutoff to predict lack of gene amplification. This was validated by studying an additional 334 consecutive cases. See table.

Conclusions: Before an IHC assay is put into clinical practice it should be validated. Fractional scoring of IHC by image analysis allows the pathologist to assess the level of protein expression, plot its distribution and optimize cutoff levels.

	FISH -	FISH +
Maximum score< 1.9	256	1
Maximum score > 1.9 but Average score < 2.4	23	10
Average score > 2.4	0	44

240 A system to estimate relapse-free survival probabilities and probable benefit from adjuvant therapy in patients with operable breast cancer.

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Background: Estimates of Relapse-free survival (RFS) in early stage breast cancer are usually based on univariate analyses and multi-variate linear models that include a variety of prognostic factors. Such models yield relatively few sub-groups limiting prognostic precision and assume proportional hazards.

Materials and Methods: Proportional hazards models, using fractional polynomial for the continuous variables, were derived from 3083 pts with operable breast cancer treated at Guy's Hospital between 1975-1999. From the final multivariable model a 66-point scoring system, Guy's Risk Score (GRS), was developed. Each GRS point represents a 10% rise in the relative risk of an event for RFS.

Results: The final model contained 6 factors and their contributions, expressed as a percentage of the range, were nodal status 33%, tumour size 21%, grade 15%, age 12%, hormonal therapy and oestrogen receptor +ve disease 11% and chemotherapy 6%. The 5-, 10- and 20-year RFS probabilities were respectively 96%, 91% and 80% in the best prognostic group (GRS ≤12, [5% of pts]) and 12%, 5% and 3% in the worst group (GRS ≥ 42, [3% of pts]). The effect of hormonal therapy was highly significant in the ER+ subgroup only (hazard ratio = 0.5) and use of chemotherapy was associated with a reduction in risk of relapse (hazard ratio = 0.7).

Discussion: A model has been derived, to provide accurate estimates of relapse-free survival from established prognostics factors. The model provides a wide range of scores. Thus for an individual patient precise estimates, with small confidence intervals, of relapse-free survival can be obtained. The technical improvements can largely be attributed to the use of the fractional polynomials that have divided the previously used sub-groups into either individual values or smaller sub-groups that allows more weight to be given to small changes in the prognostic factors. Information from this single data set also attributes scores to the use of adjuvant systemic therapy which result in changes in relapse-free survival which are consistent with the estimates from the EBCTCG Overview. Prognostic scores could be printed on prepared forms and a patient's score easily calculated and the associated probabilities read from tables.

241 Preoperative core-needle biopsy might affect the plasminogen activator inhibitor (PAI-1) tumor content detected by ELISA in human breast carcinomas, thus causing false positive results.

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Background: The prognostic impact of the proteolytic factors uPA (urokinase plasminogen activator) and its type-1 inhibitor (PAI-1) in node negative breast cancer has been confirmed in various clinical studies. Core-needle biopsy play an increasing role for preoperative diagnosis of breast cancer. As PAI-1 is involved in wound repair mechanisms, we examined whether the wound caused by the core-needle might induce PAI-1 expression in breast cancer tissue.

Material and Methods: Urokinase plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) were detected by an enzyme-linked immunosorbent assay in tissue extracts from 350 patients with primary breast cancer, treated at our hospital. Core needle biopsies were done in 39 cases. In additional cases, tumor tissue specimens were collected separately from areas adjacent to the biopsy channel and from areas in more than 5 mm distance within the tumors.

Results: The tissue content for PAI-1 was significantly higher in tumors from patients which underwent preoperative core-needle biopsy: (Median 21,9 vs 13,6 ng/mg protein; p=0,009). No difference could be seen for uPA levels (Median 3,3 vs 2,9 ng/mg protein) There was no relation between the core biopsy and any other factor like grading, tumorsize, hormone receptor status. When applying the generally recommended cut-off level for PAI-1, the percentage of high-risk patients was 69% in the core biopsy group compared to 55% in the non core biopsy group. The median tumor tissue content for PAI-1 in specimens adjacent to the biopsy channel was 4 fold higher than those from distant tumor areas.

Discussion: Our data indicates that core-needle biopsy might affect the detection of PAI-1 in tumor tissue. We strongly recommend not to collect tissue specimens from tumor areas adjacent to core-needle channels within the tumor. Data from patients that underwent core-needle biopsies should be considered with great caution with regard to their prognostic information.

242 Breast ductal carcinoma in situ: nuclear grading by discriminant analysis of image features.

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Background: Pathologically determined nuclear grade is a major determinant in the therapeutic management of patients with breast carcinoma in situ (DCIS). However, nuclear grades are difficult to distinguish reproducibly, and patients with mixed grades present additional problems. We are working on the development of quantitative nuclear grades using computer-aided image analysis.

Materials and Methods: Specimens of 82 patients, stained with H&E, were graded by a breast pathologist according to the Van Nuys system. For each patient, up to 20 nuclei images were acquired with a monochrome CCD camera and values for 39 features were extracted that quantified size and shape, amount of DNA, and arrangement of DNA in the nucleus. Fisher linear discriminant analysis was used to determine features that group patients into Van Nuys grades.

Results: Discriminant functions distinguished Van Nuys grades with correct jackknifed classification for 62.5% of slides with grade 1 and mixed grades 1 and 2, 38.9% of pure grade 2, 42.9% of mixed grades 2 and 3, and 65.2% of pure grade 3 DCIS. Plots of the first 2 canonical variables show a clear progression across the ordered pathologic grading groups.

Discussion: Nuclear features, measured by image analysis, may provide quantitative functions that distinguish between Van Nuys pathologic grades of DCIS. Classification improvements are expected with the acquisition of additional nuclei for each patient.

243 Estrogen receptor heterogeneity in breast cancer objectively quantified by QCA imaging system.

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Background: Heterogeneity of estrogen receptor (ER) expression likely plays an important role in breast cancer behavior and responsiveness to chemotherapy. However, means to objectively quantify such heterogeneity have been mostly unavailable, and the current bimodal positive vs. negative reporting standard ignores tumor heterogeneity as a potentially important factor. Even the reporting of percent positivity ignores variability in staining intensity that reflects cellular differences in protein expression.

Materials and Methods: We therefore developed a new image analysis system (QCA) to provide automated measurements of immunohistochemistry special-stained tissue section slides. The system comprises software that accomodates to most light microscope, video camera, computer environments. Images are selected and captured by a pathologist from the glass slide, and analysis can be fully automatic or manual as desired. Results are depicted in three forms: graphically, statistically, and via a colorized image. The graphical result is a frequency histogram of stain intensity. Statistical results include a cumulative score, a heterogeneity score, and percent positivity at different levels of intensity. Using the QCA system, 50 breast cancer cases from the University of Florida were each analyzed by two different pathologists. The specific goal was to establish the reproducibility of the imaging system, particularly for the heterogeneity score measurement.

Results: The QCA system showed that the 50 breast cancers exhibited a surprisingly wide range of ER heterogeneity, with 42 (84%) of the cancers comprising at least some completely negative and some strongly ER positive tumor cells. Moreover, the reproducibility of the heterogeneity scores was good, with regression between pathologists yielding r = 0.82.

Discussion: ER heterogeneity may well prove to be another important indicator in defining patient outcome, and/or in determining which breast cancer patients may respond best to ER blockers or estrogen synthesis inhibitors. Our initial studies show that the QCA image analysis system provides an objective means of defining heterogeneity for ER or other relevant antigens. Further studies are now in progress to expand the data and correlate the heterogeneity results with clinical indicators of patient outcome.

244 Interobserver agreement for estrogen receptor quantitation by manual and automated scoring.

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Background: Standardized testing for estrogen receptor (ER) is lacking and the reliability of immunohistochemistry (IHC) for measuring ER has recently been questioned. In addition to pre-analytical variables, and interlaboratory differences in performing IHC, scoring appears to be a significant source of variability. Scoring practices vary widely throughout the United States and internationally and include semi-quantitative scoring formulas, manual estimations, and computer-assisted techniques. Our goal was to determine the rate of interobserver variability for manual ER scoring at our institution by IHC and compare the results to ER scores obtained using the Quantitative Image Analysis software (QCA2, Lake Forest, IL).

Material and Methods: Using the monoclonal antibody 6F11, ER was assayed on 41 consecutive formalin-fixed, paraffin-embedded invasive breast cancers by standard IHC techniques. Scoring was performed independently by 3 breast pathologists. The percentage of positive invasive tumor cells were recorded by each observer and an intensity score was not used. The following scale was utilized: 0%: negative, 1%-9%: negative (low expressors), 10% or greater: positive. Additional analysis of the cases using the QCA2 software package was performed. We calculated a concordance rate for the positive results and kappa scores were determined using the Cohen coefficient.

Results: 28 of 41 cases (68%) were found to be ER+ (range 20%-100% of nuclei) and 12 cases (29%) were ER negative. A single case (2%) showed ER staining of less than 10%. The concordance rate between manual ER scoring and image-analysis was 89%. The consensus scores for the 41 cases had high strengths of agreement with the ER scores determined by image analysis (kappa=0.91). Interobserver variability was found to be low and the kappa score of each observer showed strong agreement when compared to the consensus score, the image analysis score, and between the observers (lowest kappa=0.84).

Discussion: Our results demonstrate that interobserver agreement for manual scoring of ER is very strong for breast cancers assayed using IHC and that manual or computer-aided scoring techniques are comparable as determined by kappa score analyses.

245 Endocrine therapy combined with the farnesyl transferase inhibitor (FTI) R115777 produces enhanced tumor growth inhibition in hormone-sensitive MCF-7 human breast cancer xenografts in-vivo.

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Background: We have shown that the FTI R115777 delays growth of hormone-dependent MCF-7 xenografts, and that R115777 induces tumor regressions and stable disease ≥24 weeks in 25% patients with advanced breast cancer. Preclinical data suggest that FTIs may be cytostatic in some systems, but apoptosis may be induced by withdrawal of serum or blockade of the PI3K pathway. Tamoxifen (Tam) or estrogen (E2) withdrawal modulates the insulin-like growth factor pathway which activates PI3K/AKT. We hypothesised that combining FTI with Tam or E2 deprivation may induce greater tumor regressions than either treatment alone.

Materials and Methods: MCF-7 xenografts were established in oophorectomised mice and randomised to 19 days treatment with either: A) continued E2 support alone, B) E2 + FTI 50mg/kg bid, C) E2 + Tam 20mg/kg od, D) E2 + Tam 20mg/kg od + FTI 50mg/kg bid, E) withdrawal of E2 support, or F) withdrawal of E2 support + FTI 50mg/kg bid.

Results: Combined therapy with Tam + FTI produced greater inhibition of E2 dependent tumor growth vs either Tam ($p=0.041$) or FTI ($p<0.001$). While Tam induced tumor stasis (mean relative tumor volume, MRTV 1.9+/-0.43 SEM), and FTI had only a modest effect vs E2 controls (MRTV 4.85+/-0.37 vs 5.06+/-0.86), Tam + FTI induced clear tumor regressions (MRTV 0.7+/-0.19). Likewise, E2 deprivation + FTI induced greater growth inhibition vs either E2 deprivation ($p=0.03$) or FTI ($p<0.001$). Xenograft cell proliferation (Ki67) at day 18 was lowest in Tam + FTI treated tumors vs Tam or FTI (mean Ki-67 scores 5.0% vs 16.9% and 67.3% respectively), and in E2 deprivation + FTI treated tumors vs E2 deprivation (2.2% vs 7.6%, $p=0.029$). No differences were seen in apoptosis scores between the groups. ER, MAPK and PI3K/Akt pathways are being analysed.

Discussion: These data suggest combining R115777 with endocrine therapy enhances tumor growth inhibition in hormone dependent breast cancer xenografts compared with either therapy alone, and support design of a randomised clinical trial in advanced breast cancer.

246 Blockade of the estrogen receptor/growth factor cross-talk implicated in breast cancer tamoxifen resistance using a selective EGFR TK inhibitor.

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Evidence suggests that cross-talk between the estrogen receptor (ER) and the epidermal growth factor receptor (EGFR)/HER2 pathways influences endocrine response and resistance in breast cancer. Indeed, using a xenograft model, we have shown that HER2 overexpression increases the agonist properties of tamoxifen (tam), resulting in stimulated growth as a mechanism of de novo resistance. ZD1839 is a selective EGFR tyrosine kinase inhibitor that blocks signaling through EGFR and HER2 (following heterodimerization). We therefore investigated how cross-talk between ER and EGFR/HER2 contributes to the agonist activity of tam and whether ZD1839 could overcome endocrine resistance. Human ER-positive MCF-7 breast cancer cells were compared with a derivative line engineered to overexpress HER2 (HER2-18), which is now stimulated by tam. In both cell lines, induction of EGFR/HER2 signaling by EGF and heregulin (HRG) activates the downstream kinases AKT and p42/44 MAPK and phosphorylates both ER and its coactivator AIB1. All these effects are fully blocked by ZD1839. Estradiol (E2)-induced ER phosphorylation is not blocked by ZD1839. Notably, however, in the HER2-18 cells, ZD1839 attenuates tam-induced ER phosphorylation, and completely inhibits both E2- and tam-induced phosphorylation of HER2, AKT and p42/44 MAP kinase. Using transient transfections and Western blot analysis of known endogenous ER-dependent genes, such as progesterone receptor, hsp27, and bcl-2, we found that, in the HER2-18 cells, tam is almost as good an inducer as E2, probably as a result of high phosphorylation of ER and its co-accessory proteins in these cells. Importantly, this agonist activity of tam was blocked by ZD1839, thus linking the inhibitory effects of ZD1839 to its effects on ER-dependent transcription. These studies demonstrate that ZD1839 blocks cross-talk between ER and EGFR/HER-2 signaling pathways, and provide further evidence that combining ER-targeted therapy with EGFR/HER2 inhibitors is a promising new strategy that should be tested in the clinical setting.

247 The estrogen receptor antagonist fulvestrant, but not the partial antagonist tamoxifen, is an effective endocrine treatment in a xenograft model of HER2 overexpressing breast cancer.

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Experimental and clinical evidence suggests that cross-talk between the estrogen receptor (ER) and the epidermal growth factor (EGFR)/HER2 pathways contributes to endocrine therapy resistance. Using human ER-positive breast cancer cells engineered to overexpress HER2 (MCF-7/HER2-18), we have shown that HER2 overexpression increases the agonist properties of tamoxifen, resulting in stimulated growth as a mechanism of de novo resistance. Fulvestrant (Faslodex) is an ER antagonist that downregulates ER which was recently approved for the treatment of metastatic breast cancer resistant to tamoxifen. The effect of fulvestrant on the growth of established MCF-7/HER2-18 tumors in nude mice was determined after supplemental estrogen was removed. In contrast to tamoxifen, which stimulated growth, treatment with fulvestrant completely inhibited tumor growth. However, while in MCF-7 tumors with normal levels of HER2, fulvestrant-induced growth suppression is durable (>6 months), acquired resistance to fulvestrant in MCF-7/HER2-18 tumors developed more rapidly (2-3months), suggesting that activation of the HER2 pathway may mediate acquired resistance to fulvestrant. ZD1839 ('Iressa') is an EGFR tyrosine kinase inhibitor that blocks signaling through EGFR and HER2 in breast cancer cells. To investigate whether ZD1839 can delay the onset of fulvestrant resistance in HER2-overexpressing tumors, mice bearing MCF-7/HER2-18 tumors were treated with fulvestrant either alone or in combination with ZD1839. In mice given continued estrogen supplementation, ZD1839 only modestly delayed tumor growth. When combined with fulvestrant, however, ZD1839 resulted in a significant and marked delay in tumor progression, implicating a major role for the EGFR/HER2 pathway in acquired resistance to fulvestrant. These studies show that fulvestrant, unlike tamoxifen, can effectively inhibit growth of ER positive HER2-overexpressing breast cancer, and provide further evidence that combining ER-targeted therapy and EGFR/HER2 inhibitors is a promising new strategy for the treatment of breast cancer that should be tested in the clinical setting.

'Faslodex' and 'Iressa' are trademarks of the AstraZeneca group of companies.

248 The epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 ('Iressa') enhances the antitumor effect of the antiestrogen ICI 182,780 ('Faslodex') in estrogen-receptor-positive breast cancer cells.

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Background: Epidermal growth factor receptor (EGFR) is expressed at high levels in at least 20% of breast cancers. It has been suggested that this high expression leads to a shorter survival time and resistance to endocrine therapy in patients with breast cancer. ZD1839 ('Iressa') is an orally active, selective EGFR tyrosine kinase inhibitor (EGFR-TKI) that blocks signal transduction pathways implicated in proliferation and survival of cancer cells. To test the hypothesis that inhibition of the EGFR signaling pathway enhances the antitumor effect of endocrine therapy, ZD1839 and an estrogen receptor antagonist with no known agonist activity, ICI 182,780 ('Faslodex'), were administered to human breast cancer cells.

Materials and Methods: A total of five human breast cancer cell lines (three estrogen receptor [ER]-positive and two ER-negative) were used. The expression levels of the EGFR/HER family members were measured by multiplex RT-PCR and Western blotting. The effects of single or combined treatments with ZD1839 and/or ICI 182,780 on cell growth, cell cycle progression and apoptosis were analyzed.

Results: All five cell lines tested were modestly sensitive to ZD1839, regardless of the EGFR expression levels; ZD1839 induced a G1-S blockade and apoptosis. The 50% growth inhibitory concentrations of ZD1839 were 10-15 μM. Although ZD1839 did not enhance the antitumor effect of ICI 182,780 under estrogen-deprived conditions, it did so in all three ER-positive cell lines in a medium supplemented with 1 nM estradiol. The combined treatment also enhanced cell cycle retardation and apoptosis.

Discussion: These results suggest a synergistic interaction of the EGFR-TKI ZD1839 with the antiestrogen ICI 182,780 in ER-positive breast cancer cells. These findings support the above hypothesis that inhibition of the EGFR signaling pathway enhances the antitumor effect of endocrine therapy. More detailed mechanisms of action will be discussed.

'Faslodex' and 'Iressa' are trademarks of the AstraZeneca group of companies.

249 Fulvestrant ('faslodex') as hormonal treatment in postmenopausal patients with advanced breast cancer progressing after treatment with tamoxifen and aromatase inhibitors.

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Background: Fulvestrant ('Faslodex'; formerly ICI 182,780) is a new class of antiestrogen that is completely free of agonist activity. In postmenopausal (PM) women with advanced breast cancer (ABC) progressing on prior endocrine therapy, it has shown activity similar to the aromatase inhibitor (AI) anastrozole (A. Howell. Eur J Cancer 2001;37 [Suppl. 6]:151;Abstr.550). This ongoing trial conducted by SAKK, aims to assess the efficacy of fulvestrant as third-line treatment in ABC patients (pts) progressing after tamoxifen and AIs. **Materials and Methods:** This is a phase II, open, multicenter, non-comparative study recruiting PM pts with ABC who had received prior endocrine treatment with tamoxifen and AIs. After enrolment of 21 pts, inclusion criteria were modified to include pts progressing after steroidal AIs (in addition to non-steroidal). **Results:** We report on the first 20 pts followed for at least 6 months. Median age was 67 years (range 45-86). The majority of pts had bone metastases (14 pts); 9 pts had liver metastases; skin metastases were seen in 4 pts and lymph node metastases were seen in 3 pts. Two pts had lung metastases and 3 pts had breast metastases. Twelve pts received prior chemotherapy, 5 of them had one line of chemotherapy for metastatic disease. All pts received anastrozole or letrozole as second-line treatment for ABC except 2 pts who received an AI as first-line treatment after progression on adjuvant tamoxifen. Two pts showed a partial response (PR) and 5 pts had stable disease (SD) for ≥24 weeks, which represents 41% of the eligible pts. Three pts were ineligible and 10 pts showed a progressive disease. Further update of response rate and tolerability will be presented. Fulvestrant was well tolerated: side effects were mild (grade 1 and 2) and comprised fatigue in 3 pts, chills in 3 pts, nausea and vomiting in 3 pts, constipation in 2 pts, hot flashes in 2 pts and stomatitis in 2 pts. **Discussion:** In this heavily pre-treated population, a clinical benefit with fulvestrant (PR or SD ≥24 weeks) was observed in 41% of eligible pts after tamoxifen and AI failure. Fulvestrant is a potential alternative treatment option for PM women with ABC, progressing on AIs.

250 Second generation of estrogen receptor destabilizing compounds-pharmacological profile in antiestrogen-sensitive or -resistant breast cancer.

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The principal limitation of selective estrogen receptor modulators (SERM) in the treatment of hormone-sensitive breast cancer is due to their limited antiestrogenic specificity reflected by a mixed agonist/antagonist profile. The early onset of resistance or loss of activity might also be related to this matter. A drug finding program on pure antiestrogens led to the identification of a series of new and extremely potent compounds. These new antiestrogens belongs to the class of specific estrogen receptor destabilisators (SERD) and are characterized to be highly active and specific pure estrogen receptor antagonists which destabilize the estrogen receptor protein most efficiently. These steroidal compounds, moreover proved to have a strong inhibitory effect on estrogen-stimulated uterine growth in mice and rats and no agonistic activity could be found.

Data obtained in several preclinical tumor models in mice and rats showed a impressive intrinsic potency in growth inhibition of estrogen receptor positive breast cancer. Compared to established therapeutic regimes, a 3-4 fold prolongation of effective tumor growth control (time-to-relapse) was found in MCF-7, ZR-75, and T47D breast cancer models. Even more important, we were able to show that the new pure antiestrogens prevented further tumor progression in several tamoxifen resistant breast cancer models. Growth inhibition of tamoxifen resistant MCF-7 and ZR-75 human breast cancer xenografts was shown not to be only transient. The observed long-lasting effect was comparable to that in native antiestrogen sensitive models. Mechanistic studies revealed the complete down-regulation of the estrogen receptor in human breast tumors as the most likely reason for the superior experimental tumor growth inhibition.

Furthermore, we have compared the new antietrogens with the first generation SERD Faslodex™. Increased potency and efficacy following oral administration provide a major advantage. This new generation of pure antiestrogens, therefore may provide a significant clinical progress for the endocrine treatment of hormone-sensitive breast cancer.

251 Postmenopausal women with advanced breast cancer who progress on fulvestrant or tamoxifen retain sensitivity to further endocrine therapies.

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Background: Fulvestrant (Faslodex, FAS) is an estrogen receptor (ER) antagonist that downregulates the ER and has no known agonist activity. The efficacy and tolerability of FAS has been compared with that of tamoxifen (TAM) in a randomized, multinational, double-blind trial involving postmenopausal women with metastatic or locally advanced breast cancer (ABC). **Materials and Methods:** Patients (pts) who were previously untreated for ABC were randomized to receive either FAS (250 mg, i.m. injection, once monthly; n=313) or TAM (20 mg, orally, once daily; n=274). A follow-up questionnaire was used to gather data on subsequent therapy from all pts. Presented here are data from pts who initially derived clinical benefit (CB; complete response, CR; partial response, PR; stable disease ≥ 24 weeks, SD) on FAS or TAM and who, after disease progression, received further endocrine therapy, and whose responses to subsequent therapy were also available. **Results:** A total of 66 pts (35 FAS- and 31 TAM-treated patients) received subsequent endocrine therapy and fulfilled the above criteria. The majority of pts were treated with an aromatase inhibitor (AI; anastrozole [AN] n=16; letrozole [LET] n=5; fadrozole [FAD] n=1) after FAS. This resulted in a CR in 1 pt, a PR in 1 pt, SD in 9 pts and progression in 11 pts (CB rate of 54%). Ten pts received TAM after FAS, which produced a PR in 1 pt, SD in 7 pts and progression in 2 pts. Three pts received a progestin, of which 1 pt experienced SD and 2 pts progressed. Similarly, after progression on initial TAM, the majority of pts were treated with an AI (AN n=15; LET n=4; FAD n=2; exemestane n=3), which resulted in a CR in 2 pts, a PR in 1 pt, SD in 13 pts and progression in 8 pts (CB rate of 67%). The remaining pts received a progestin (n=6) or FAS (n=1) after initial TAM, which resulted in SD in 3 pts and progression in 4 pts. Overall, endocrine therapy after first-line FAS produced CB in 20/35 (57%) pts. CB with subsequent endocrine agents was also achieved in 19/31 (61%) pts after first-line TAM. **Discussion:** FAS is effective in pts progressing on TAM and these data show that after receiving FAS, pts remain sensitive to treatment with other endocrine agents, including TAM and AIs. FAS therefore provides an additional treatment option that may extend the period of effect of endocrine therapy, before cytotoxic chemotherapy needs to be considered.

252 Continuous vs intermittent tamoxifen (T) versus intermittent / alternated T and medroxyprogesterone acetate (MPA) in advanced breast cancer. An EORTC phase 3 study (trial 10863).

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Background: This study was based on the hypothesis that total elimination of endocrine responsive cells in heterogenous breast cancer could accelerate the development of hormone independent cell clones.

Patients and methods: Postmenopausal patients with possible hormone responsive advanced breast cancer responding at 4 months to first line T (40 mg daily) were randomized to continuous or intermittent T (2 months break, two months treatment etc.) or to intermittent / alternated T and MPA (300 mg daily). Response was evaluated every 2 months. If progression occurred during break or MPA, patients received T continuously. Endpoints of the study were time to proven resistance to T, and progression free and overall survival.

Results: Between 1987 - 1996, 593 patients were registered. 44% had an unknown, 56% a positive receptor status. Of 347 (58%) initial responding (CR, PR, NC) patients, 276 were randomized. Patient characteristics in the treatment arms did not differ significantly. After a median follow-up time of 8 years the median times to resistance to T were 12.2, 12.4 and 23.3 months for T, intermittent T and T / MPA respectively ($p<0.001$). Although time between disease progression during break or MPA and subsequent progression during continuous tamoxifen exceeded the interval time of 2 months in individual patients, this was not translated in significant differences in median time to progression or survival, partly due to bias by protocol construction. The median survival times were 36.1, 36.1 and 32.1 months, respectively.

Discussion: Intermittent / alternated T and MPA resulted in a significant prolonged time to proven resistance to T. However, intermittent T or intermittent / alternated T and MPA did neither prolong, nor shorten the times to progression or death as compared with classical continuous T in patients with advanced breast cancer.

253 Insulin receptor substrate-1 levels correlate with estrogen receptor status and are decreased following neoadjuvant anti-estrogen treatment of postmenopausal breast cancer.

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Background: Insulin receptor substrate-1 (IRS-1) is a cytoplasmic signal transduction molecule involved in insulin-like growth factor (IGF) signaling. We have previously shown that IRS-1 is an estrogen-regulated gene in the normal murine mammary gland, and human breast cancer cell lines and xenografts. Furthermore, an immunoblot study of IRS-1 levels in axillary-node negative breast cancer patients (n=200) indicated that IRS-1 levels correlated weakly with estrogen receptor (ER) (r=0.15, p=0.03) and progesterone receptor (PR) status (r=0.12, p=0.09). In the ER-positive patients (n=147), high levels of IRS-1 (above the median level) conferred significantly reduced disease-free survival (p=0.05).

Goal: To test if IRS-1 levels correlate with ER status by immuno-histochemistry (IHC) and if IRS-1 levels are lowered by neoadjuvant anti-estrogen therapy.

Materials and Methods: IRS-1 antibody was optimized for IHC on paraffin sections and specificity confirmed using multiple different controls. IRS-1 was examined in two sets of clinical samples. First, IRS-1 was measured in a bank of primary breast cancer specimens (n=242). Second, IRS-1 was measured in core biopsies (0, 2, and 12 weeks) from postmenopausal primary breast cancer patients randomized to treatment presurgically with tamoxifen (20mg per day, n=17) or vorozole (2.5 mg per day, n=16).

Results: In primary breast cancer specimens IRS-1 was heterogeneously expressed, and similar to the previous immunoblot study, there was a trend for IRS-1 to correlate weakly with ER (r=0.12, p=0.07) and PR (r=0.10, p=0.11). In presurgical biopsies of postmenopausal breast cancer patients before preoperative hormone therapy, IRS-1 levels were again heterogeneously distributed. Overall treatment resulted in a significant decrease in IRS-1 levels with tamoxifen (p=0.0006) and a trend to a decrease with vorozole (p=0.086). This was seen after 2 weeks of tamoxifen compared to pretreatment (p=0.007), and also seen at 12 weeks (p=0.004). No significant change was seen between 2 weeks and 12 weeks of treatment.

Discussion: We have shown here that IRS-1 correlates weakly with the ER and PR status of breast cancer patients, and that presurgical treatment of postmenopausal breast cancer with tamoxifen significantly reduces IRS-1 levels. The suppression of plasma IGF-I levels by tamoxifen but not vorozole (Harper-Wynne C.L., 2002 J Clin Oncol; 20:1026-35) may have influenced the trend to a greater change in IRS-1 levels with the antiestrogen. These data would support preclinical studies suggesting that one mechanism of anti-estrogen action may be to downregulate the IGF signaling pathway.

254 Updated survival results from the Zebra trial.

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Background: The Zoladex Early Breast cancer Research Association (ZEBRA) trial was initiated to compare the efficacy and tolerability of goserelin with cyclophosphamide, methotrexate, 5-fluorouracil chemotherapy (CMF) in pre- and perimenopausal women with node-positive early breast cancer.

Methods: Pre- and perimenopausal patients (pts) [≤50 years of age] entered the trial and received either goserelin (3.6 mg every 28 days for 2 years, n=817) or CMF (6 x 28-day cycles, n=823) for the adjuvant treatment of breast cancer. Initial results showed a highly significant interaction between treatment and estrogen receptor (ER) status, with equivalent benefit of goserelin and CMF in pts with ER-positive tumors but superiority of CMF in pts with ER-negative tumors. Here we present an update from the ZEBRA trial in which pts were followed-up for a median of 7.3 years.

Results: In pts with ER-positive tumors, goserelin (n=591) continued to be equivalent to CMF (n=598) for disease-free survival (281 vs 269 events; hazard ratio [HR] = 1.05; 95% confidence interval [CI] 0.88, 1.24). Median disease-free survival was 7.9 years in the goserelin group and 8.0 years in the CMF group. In pts with ER-positive tumors, at the time of this analysis there was a total of 148 deaths in the goserelin group (25.0% pts) and 154 deaths in the CMF group (25.8% pts). Non-inferiority of goserelin vs CMF was shown by the HR (0.94) and 95% CI (0.75, 1.18) for overall survival. In pts with ER-negative disease, goserelin (n=144) was inferior to CMF (n=160) for disease-free survival (HR = 1.83; 95% CI 1.33, 2.52) and for overall survival (HR = 1.64; 95% CI 1.13, 2.39).

Discussion: This extended follow-up analysis demonstrates that goserelin continues to show equivalence vs CMF for disease-free survival in ER-positive pts. With equivalent efficacy vs CMF and proven quality-of-life benefits, goserelin therefore offers an effective alternative to CMF chemotherapy in the management of pts with ER-positive, node-positive early breast cancer.

255 Anastrozole ('arimidex') versus tamoxifen as first-line therapy in postmenopausal women with advanced breast cancer: Results of the double-blind crossover SAKK Trial 21/95 - a sub-study of Anastrozole Trial 0027.

Thuerlimann B, Hess D, Koeberle D, Senn I, Ballabeni P, Pagani O, Perey L, Aebi S, Rochlitz C, Goldhirsch A. (SAKK), Berne, Switzerland

Introduction: Combined analysis of two randomized double-blind trials (0027 and 0030) showed that anastrozole (ARI) has efficacy and tolerability advantages over tamoxifen (TAM) as first-line treatment of postmenopausal women with advanced breast cancer (ABC). A questionnaire retrospectively administered to investigators revealed, based on unblinded data, that ARI was effective after TAM and vice-versa. **Methods**: Within trial 0027, all patients randomized in Swiss centers were followed through both the first-line therapy and the subsequent crossover phase, maintaining treatments blinded. Here we present these results for the first time. **Results**: 60 patients (ARI n = 31; TAM n = 29) were recruited in the SAKK 21/95. Median age was 68 years (47-85). Median weight was 67 kg (42-96). Estrogen and/or progesterone receptors (ER/PgR) were positive in 56 and unknown in 4 patients. Most frequent sites of disease were bone (45), lung (28), skin (24) and nodes (17). Median time to progression (TTP) on first-line therapy for 60 randomized patients was 11.3 months and 8.3 months for ARI and TAM, respectively (log-rank test: p=0.75). After progression 19 patients switched from ARI to TAM and 18 patients switched from TAM to ARI. Median TTP on first-line therapy for the 37 patients with subsequent crossover was 16.3 and 5.9 months. Median TTP was 6 months for both second line treatments (ARI after TAM and TAM after ARI). Median time from randomization to second progression was 28.2 months for the sequence ARI-TAM and 19.5 months for TAM-ARI (p=0.36). **Conclusions**: Both treatment sequences have shown clinically relevant efficacy. In patients with endocrine-responsive disease and indication for second-line endocrine therapy, ARI showed longer TTP than TAM when given as first-line therapy. After crossover, both ARI and TAM have similar activity. These results are supportive of the recommendation to give ARI as first-line treatment for ABC in postmenopausal women with ER and/or PgR-positive disease.

256 Significantly longer time to progression for Femara® (letrozole) in patients with or without prior adjuvant tamoxifen: updated analysis of the double-blind, randomized, multinational phase III trial of letrozole compared to tamoxifen as first-line hormonal therapy for advanced breast cancer.

Mouridsen H, Sun Y, Gershanovich M, Perez-Carrion R, Chaudri-Ross HA, Lang R. Rigshopitalet, Copenhagen, Denmark; Chinese Academy of Medical Sciences, Beijing, China; Petrov Research Institute of Oncology, St. Petersburg, Russian Federation; Hospital Universitario de la Princesa, Madrid, Spain; Novartis Pharma AG, Basel, Switzerland

Background: Letrozole Femara® 2.5 mg has demonstrated superior efficacy over tamoxifen 20 mg in the first-line treatment of postmenopausal women with advanced breast cancer and reduced the risk of progression by 30% compared with tamoxifen (J Clin Oncol 2001;19(10):2596-606). An updated analysis of the trial was reported in December 2001 at a median follow-up of 32 months. The primary endpoint TTP (time to progression) was superior for letrozole compared with tamoxifen (hazard ratio, 0.72; P<0.0001), as well as ORR and CBR (odds ratio for objective response 1.78, P=0.0002, for clinical benefit 1.62, P=0.0004). These results confirmed the consistency of the first analysis. A significant survival benefit during the first 2 years of therapy in favor of letrozole was reported (P<0.02).

Methods: We here report the impact of prior adjuvant tamoxifen therapy on TTP, ORR and CBR.

Results: Patients who received no prior adjuvant tamoxifen had a median TTP of 9.5 months (mos) in the letrozole arm (n=369) vs. 6.0 mos in the tamoxifen arm (n=371); P=0.0003. In those patients who received adjuvant tamoxifen, the median TTP for letrozole was significantly longer (P=0.0033) at 8.9 months (n=84) than for tamoxifen at 5.9 months (n=83). ORR was significantly higher for letrozole in both groups (no adjuvant tamoxifen: 33% vs 24%, P=0.0039; adjuvant tamoxifen: 26% vs 8%, P=0.0038). CBR was significantly higher for letrozole in both groups (no adjuvant tamoxifen: 51% vs 40%, P=0.0026; adjuvant tamoxifen: 46% vs 31%, P=0.0464).

Discussion: These data demonstrate that both tamoxifen-naïve and those patients who received adjuvant tamoxifen have a significantly longer TTP and significantly higher rates of response and clinical benefit when treated with letrozole. These data further demonstrate the consistent superiority of letrozole vs. tamoxifen, and support the use of letrozole as a new standard of hormonal therapy in postmenopausal women with advanced breast cancer.

257 Time to progression and tumor response according to estrogen receptor status in the international phase III study of letrozole (Femara®) and tamoxifen as first-line hormonal therapy for advanced breast cancer.

Monnier A, Sun Y, Gershanovich M, Perez-Carrion R, Chaudri-Ross HA, Lang R. CHG Andre Boulloche, Monbeliard, France; Chinese Academy of Medical Sciences, Beijing, China; Petrov Research Institute of Oncology, St. Petersburg, Russian Federation; Hospital Universitario de la Princesa, Madrid, Spain; Novartis Pharma AG, Basel, Switzerland

Background: Letrozole (Femara®) 2.5 mg demonstrated superior efficacy over tamoxifen (TAM) 20 mg in the largest double-blind, randomized, international phase III trial that was the basis for its approval as first-line treatment of postmenopausal women with advanced breast cancer (J Clin Oncol 2001;19(10):2596-606).

Methods: We report here the prespecified analysis of tumor endpoints by receptor status, conducted at a median follow-up of 32 months.

Results: As summarized in the table, the results by receptor status were consistent with the results of the study as a whole. Multivariate analysis indicated that neither ER nor PgR alone significantly influenced any tumor endpoint, although longer TTP and higher rates of response and clinical benefit were obtained when both receptors were positive.

Receptor status	Endpoint	Statistic	LET	TAM	P-value
ER+ and PgR+		n	174	186	
	TTP (m)	Median	11.9	6.0	<0.0001
	Response	Rate (%)	37	21	0.0007
	Clinical benefit	Rate (%)	57	41	0.0025
ER+ and/or PgR+		n	294	305	
	TTP (m)	Median	9.4	6.0	<0.0001
	Response	Rate (%)	33	22	0.0019
	Clinical benefit	Rate (%)	51	40	0.0069
Unknown*		n	159	149	
	TTP (m)		9.2	6.0	0.0408
	Response	Rate (%)	30	19	0.0309
	Clinical benefit	Rate (%)	48	34	0.0160

*Includes 4 pts with negative status assigned to LET

Discussion: The superiority of letrozole (LET) over TAM in tumor endpoints was demonstrated in this study regardless of whether receptor status was positive or unknown. Patients with tumors positive for both steroid receptors appeared to gain longer TTP and higher response and clinical benefit rates, particularly when treated with LET.

258 Quality-adjusted survival of letrozole versus tamoxifen in post-menopausal women with advanced breast cancer.

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Background: In a randomized multicenter clinical trial first-line treatment with letrozole (2.5 mg) significantly prolonged time to disease progression compared to tamoxifen (20 mg) among post-menopausal women with advanced breast cancer (median 9.5 vs 6 months) (Mouridsen, et al. J Clin Oncol 2001;19:2596-2606).

Objective: To determine the quality-adjusted time without symptoms or toxicity (Q-TWiST) of letrozole vs. tamoxifen using the trade-off between the clinical benefits and toxicities.

Methods: Three clinical health states were defined: time with toxicity (Tox), time without disease progression or toxicity (TWiST), and time following disease progression (Prog). The toxicity state was defined as any period in which the patient experienced an adverse event of severity grade 3 or 4 regardless of attribution to study drug. Kaplan-Meier estimates of the mean amount of time spent in each health state was determined separately for each treatment group. Health state durations were calculated at 32 months median follow-up. The health states Tox and Prog were weighted using utility scores (range: 0 - 1) to account for possible decrement in quality of life relative to the health state TWiST. Q-TWiST was calculated as the weighted sum of the health state durations and the bootstrap method was used to generate variance estimates. Treatment comparisons were made for different values of the utility weights using a threshold utility analysis.

Results: The ITT population consisted of 907 patients (letrozole, N=453 and tamoxifen, N=454). We present results using utility weights for the Tox and Prog states equal to 0.5 relative to a utility of 1 for TWiST. The mean duration of Tox for letrozole was 3.5 months and 2.9 months for tamoxifen (p=0.10). For letrozole, the mean duration for TWiST was 10.1 months compared to 7.6 months for tamoxifen (p=0.0004). The mean duration of Prog for letrozole was 11.4 months and 12.6 months for tamoxifen (p=0.089). There was a 2.2-month difference in quality-adjusted survival (Q-TWiST) duration favoring letrozole over tamoxifen (17.5 vs. 15.3 months, respectively; p<0.0001).

Conclusions: Letrozole used as first-line therapy for advanced breast cancer offers patients significantly more quality-adjusted survival than tamoxifen.

259 Evaluation of the activity of letrozole, anastrozole and tamoxifen using novel three-dimensional breast cancer cell culture models.

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Two types of three-dimensional cell culture model (a spheroid culture model and a Matrigel thread model) have been used to evaluate the activities of letrozole, anastrozole and tamoxifen on the proliferation of ER-positive and aromatase-positive breast cancer cells. The three-dimensional cell culture studies were carried out using two aromatase over-expressing ER-positive breast cancer cell lines, MCF-7aro and T-47Daro. The preparation of these cell lines has been described by Sun et al. (J. Steroid Biochem. Molec. Biol., 63: 29-36, 1997). Aromatase activity in the MCF-7aro and T-47aro cell lines was determined to be 73 pmol/mg/h and 48 pmol/mg/h, respectively. For the spheroid culture, MCF-7aro and T-47Daro cell lines were cultured over a 1-mm layer of 1.5% agarose in a 100 mm Petri dish. After 48-72 hours, cell spheroids were formed, and vigorous pipetting could not separate the cells. For the Matrigel thread culture, the cells were homogeneously mixed with Matrigel solution for 10 min in ice. The cell-Matrigel mixture was delivered into a pre-chilled and sterilized 6 cm x 0.8 mm (inner diameter) Teflon tube with the aid of a 35-cc syringe. Upon exposing the Matrigel filled Teflon tube at room temperature for about 2-3 min, the congealed Matrigel was extruded out by the pressure created from the syringe. The cell spheroids or Matrigel threads were cultured in phenol red-free media containing 10% dextran-coated charcoal-treated fetal calf serum. While the two types of three-dimensional cell cultures exhibit their own unique features, the proliferation of MCF-7aro and T-47Daro, under both of the above described culture conditions, was stimulated by 1 nM 17beta-estradiol or 1 nM testosterone. Letrozole and anastrozole, two aromatase inhibitors, could suppress the testosterone-dependent proliferation, in a dose-dependent manner with letrozole being more effective than anastrozole in this respect. In contrast to the suppressive effect of the aromatase inhibitors, tamoxifen was found to act as a weak agonist of the estrogen receptor leading to a stimulation of the cell proliferation. These results demonstrate that aromatase inhibitors are more effective agents than tamoxifen thereby inhibiting the growth of hormone-responsive breast cancer cells even when grown as three-dimensional cell cultures.

260 Pharmacokinetic and pharmacodynamic interaction of adjuvant tamoxifen and exemestane in postmenopausal women treated for early stage breast cancer.

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Exemestane is a steroidal aromatase inactivator, and rat mammary cancer models suggest an additive benefit of exemestane with tamoxifen. To explore whether studying adjuvant therapy with the combination of tamoxifen (TAM) and exemestane (EXE) is reasonable, we have initiated a pilot trial to study the toxicity, pharmacokinetics, and pharmacodynamics of this combination. In this trial, postmenopausal women receiving adjuvant hormonal treatment with TAM 20mg qd as standard therapy are studied before and after an 8 week treatment with EXE, 25mg PO qd. In addition to pharmacokinetic sampling for TAM and EXE, patients are assessed for estrone, estrone sulfate, and estradiol concentrations. Area under the concentration vs time curve (AUC) over 24 hours is determined for TAM and EXE using the trapezoidal method, and PK data for the first 5 subjects are reported here; comparative hormone levels are available for 3 subjects. TAM AUC(0-24) was not significantly affected by the addition of EXE (4.27 +/- 1.61 vs 3.71 +/- 1.12 ng-hr/ml, mean+/-SD, p=0.124, paired t-test). Similarly, to date there were no significant effects of EXE upon the AUC(0-24) of the 4-hydroxy or N-desmethyl metabolites of TAM. The EXE AUC (36.65 +/- 10.26 ng-hr/ml) was lower than expected from the package insert for AROMASIN®, suggesting clearance similar to that found in normal, postmenopausal women. No significant correlations were found between estrone (E), estrone sulfate (ES), or estradiol (E2) concentrations and the plasma concentrations of TAM, EXE, or their metabolites. Concentrations of E, ES, and E2 were lower in the presence of EXE. Estradiol concentrations were undetectable in patients receiving both EXE and TAM, with E and ES concentrations averaging more than 90% lower than in those patients on TAM alone. Accrual to the planned total of 30 subjects continues.

Supported by Pharmacia.

261 Pharmacokinetic (PK) study to evaluate the combination of exemestane (E) and tamoxifen (T) in the treatment of metastatic breast cancer: preliminary results.

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E is a new Type I, steroidal aromatase inactivator that irreversibly binds to the aromatase enzyme. T has antiestrogenic and estrogenic properties and is probably the best known hormonal therapy for patients with breast cancer. The antitumor activity of E, given alone or in combination with T, was investigated in rats by Zaccheo et al (J Steroid Biochem Mol Biol 1993). The combined treatment resulted in higher antitumor activity compared to either agent alone. The increase in efficacy with this combination led us to design a pilot study in which toxicity and PK could be evaluated. Response was considered a secondary endpoint. Patients were eligible if they were postmenopausal, had either measurable or evaluable disease, or if they had prior tamoxifen and/or aromatase inhibitors either in the adjuvant or metastatic setting. Patients were not eligible if they had prior history of thromboembolic events, were on HRT, or were taking over-the-counter estrogenic supplements. Patients were given E 25 mg daily for 2 weeks. After the second week, patients continued on E and started on T 20 mg daily. Blood samples for E levels, estradiol, estrone, and estrone sulfate were collected on day 14 of the 2-week single agent E period and approximately 4 weeks after starting the combination treatment. Eighteen patients were registered in the study but only 17 underwent treatment and PK sampling. One patient withdrew consent and discontinued treatment. Median age was 62 years (range, 46-84) and all patients had a performance status of 1. Sixteen patients had received prior hormonal therapy, most of which had received more than one prior hormonal agent . Thirteen patients had received prior tamoxifen, 6 had received aromatase inhibitors (AIs), 2 toremifene, 2 progestins, and 2 had received androgens. Of the patients who had received prior AIs, 2 had previously received exemestane. All patients had estrogen and/or progesterone receptor-positive tumors. We have seen 1PR, 1MR, 3SD, 7PD, and 4 are too early to evaluate. The most common toxicity observed include: grade 1 fatigue, grade 1 / 2 hot flashes, grade 1 nausea, grade 1 vaginal bleeding, and grade 2 bone pain. PK analysis is currently being performed. Updated information, including PK analysis and estrogen levels, will be provided at a later time. The results of this study will help decide the need for further evaluation of this combination.

262 Anastrozole therapy does not compromise lipid metabolism in breast cancer patients previously treated with tamoxifen.

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Background: Tamoxifen (TAM) exerts beneficial influence on lipid profile as a result of its estrogen-like properties. A number of new-generation aromatase inhibitors used sequentially to the initial adjuvant TAM are being currently investigaed in clinical trials, including anastrozole (ANS). There are concerns, however, that ANS, as a potent supressor of estrogen, may reverse beneficial effects of TAM on the serum lipids profile. The current study updates at prolonged observation our previous results on effects of ANS used in sequence to TAM in breast cancer patients.

Materials and Methods: Analysis included 43 postmenopausal breast cancer women, converted to ANS (1mg/d.) after 14-234 weeks (median: 67) of TAM (20mg/d.) treatment used for advanced disease (N=25) or in adjuvant setting (N=18). Concentrations of basic blood lipids and body mass index values (BMI) were measured before treatment, and at minimum 24 (median: 26, range: 24 - 33) and 60 (median: 63, range: 60 - 70) weeks of ANS administration afterwards.

Results: there was no statistically significant change over time in basic lipid parametres, that included total (TCH)- (p=0.23), LDL- (p=0.26), and HDL-cholesterol (p=0.37), triglycerides (p=0.32), the number of patients with TCH≥200mg/dl (p=0.55), the atherogenic risk ratios: TCH/HDL (p=0.45) and LDL/HDL-cholesterol (p=0.39) as well as in mean BMI values (p=0.54).

Conclusion: administration of ANS for ≥ 60 months in sequence to TAM does not affect lipid profile of breast cancer patients.

263 Anastrozole demonstrates clinical and biological effectiveness in erbB2 ER positive breast cancers.

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22 postmenopausal women with large operable or locally advanced with oestrogen receptor (ER) rich breast cancers were randomised to receive 1mg or 10mg of anastrozole for 3 months following which they had surgery. The age range of women was from 56 to 92 ER levels were Allred score 5 (1 patient), 6 (3 patients), 7 (10 patients), 8 (8 patients). Responders continued on anastrozole post surgery for 5 years. Median follow up: 44 months.

All patients had erbB2 assessed in their initial biopsy using the HercepTest. Response was assessed by clinical examination (pre) and ultrasound according to standard criteria (CR/PR complete or partial response, SD stable disease). Proliferation before and after 3 months (post) was assessed by Ki67. Progesterone receptor (PgR) was assessed before and after treatment.

		Clinical		Ultrasound		Median Ki67		Fall in
ErbB2	No	CR/PR	SD	CR/PR	SD	Pre	Post	PgR
0/1+	16	15	1	10	6	23.5	5+	13/13*
3+	6	6	0	5	1	22.5	7.5~	3/4*

*5 patients PgR 0 on first biopsy, ~p=0.017, +p<0.0001

Response did not differ in relation to erbB2 status All 0/1+ and all 3+ patients had a reduction in proliferation. In the 16 patients with 0/1+ erbB2 tumours there have been 3 events (1 death, 1 local recurrence, 1 lung metastasis). In the 6 erbB2 3+ patients there have been 2 events (1 local recurrence → lung metastasis and 1 liver metastasis) both patients are still alive. These are the first data demonstrating the clinical and biological effectiveness of anastrozole in erbB2 positive ER positive breast cancers.

264 Neoadjuvant letrozole: the Edinburgh experience.

Dixon JM, Jackson J, Renshaw L, Cameron DA, Miller WR. Western General Hospital, Scotland, United Kingdon

The randomised neoadjuvant trial of letrozole versus tamoxifen reported a clinical response rate of 55% (85/154) for patients randomised to letrozole and a relationship between response and ER level.

83 postmenopausal patients with large operable or locally advanced ER rich breast cancers have been treated with 3 months of letrozole and response assessed clinically and volume changes over the 3 month study assessed by clinical measurement and ultrasound - 65 patients responded - overall response rate 78%, a significantly better response rate than in the 024 study, p=0.0004. Response rate did not differ significantly between ER categories but percentage reduction in volume did (Table).

ER Score	No of	No of	%	Median % Reduction in Tumour Volume	
Allred	Pts	Responders	Response	Clin	USS
8	60	48	80	76+	67+
6 + 7	23	17	74	63	48

+p<0.05

Letrozole is confirmed as being a highly effective agent at producing tumour shrinkage in postmenopausal women with ER rich breast cancers.

265 Examining the tolerability, quality of life and patient preference of letrozole versus anastrozole in a multicentre, randomised, single blind cross over study.

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Introduction: Although anastrozole **(A)** & letrozole **(L)** are generally well tolerated, side-effects can occur in up to 40% of women, particularly those of endocrine and gastrointestinal origin. Their use is increasing rapidly and women are taking these drugs earlier and hence longer in their treatment pathway, many having few or no symptoms of their disease. Whereas previously side effects of AI's had less relevance to quality of life (in patients with advanced malignancy), toxicity now has the greater, if not sole, contribution to their well being. It is an important humanitarian issue to know which drug is better tolerated, preferred by the majority and associated with a highest quality of life (qol).

Method: 72 postmenopausal patients (median age 67 yrs), had all previously taken Tamoxifen, gave written informed consent to be randomised in this cross over study. Analysis excluded 7 patients (1 died, 4 opted not to cross over, in 2 no questionnaires were completed). Of the remaining 65 patients, 34 (52%) received **L** 2.5mg od for 4 weeks, a six day wash out followed by **A** 1mg od for 4 weeks. The other 31 (48%) received the medication in reverse order. Clinician prescriptions were blinded but patients knew which drug they were taking. 3 patients in the 4 trial centres declined trial entry suggesting these data truly reflected routine clinical practice.

Results: Qol was significantly higher in the **L** phase versus the **A** phase measured by the Fact-es on days 1,8&28 (5.1 difference, p=0.02). On the last day of the trial, before the patient attended the clinic, an ad hoc questionnaire showed that more than twice as many women preferred to remain on **L** and than **A** (68% v 32% p<0.01), the most cited reasons being less nausea, hot flushes and GI symptoms. Women choosing **L** had significantly better qol while taking **L**, those choosing **A** had significantly better qol on **A**. A further questionnaire revealed that 92% (p<0.001) of patients welcomed the opportunity to test both drugs, to make the long-term treatment decisions themselves and recommend this to future patients.

Conclusion: The significantly better qol and tolerability of patients on **L** rather than **A** was reflected by greater patient preference for this drug, validating this user friendly trial end point. Although starting with **L** would optimise qol for many patients, giving them a choice would be better. Although more complex, patients welcomed greater opportunities to involve themselves in the decision making process to ensure they take the drug best suited to them.

266 An analysis of two contradictory, clinical trials of letrozole versus megestrol acetate, for the treatment of advanced breast cancer.

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Purpose: To interpret contradictory results of two trials, BC2 [Dombernowsky et al., J Clin Oncol 16:453-461, 1998] and P02 [Buzdar et al., J. Clin Oncol 19:3357-66, 2001], which compared two doses of letrozole (0.5 mg (L0.5) and 2.5 mg (L2.5) qd) to megestrol acetate (MA) (BC2: 160 mg once daily; P02: 40 mg qid) as endocrine therapy in post-menopausal women, with advanced breast cancer, previously treated with TAM.

Problem: In BC2, L2.5 (ORR: 24%) was significantly superior to MA (ORR: 16%), whereas in P02 it was equivalent to MA (ORR: L2.5: 16%; MA 15%). L0.5 (ORR:21%) in P02 was significantly better than L0.5 (ORR: 12%). in BC2 and L2.5 in P02 (ORR:16%).

Results: The two randomized trials differ significantly in some basic characteristics of the patient populations. Among these differences are dominant site of metastases (e.g. visceral: 48.7% in P02, 40.7% in BC2; p=0.0154), performance status (WHO grade 0: 66.3% in P02, 49.0% in BC2; p < 0.0001), receptor status category (both ER+ and PgR+: 54.3% in P02, 35.6% in BC2; p < 0.0001). The randomization in P02, performed for each country, shows big differences in outcome within various countries (e.g. ORR: Denmark: 0%; Great Britain: 36%; USA,D,CDN: 15%; p < 0.0001). The value of ORR of MA (15%) in P02 mainly comes from just one country (GB: 39%; all other countries: 11%). The equality in outcome between L2.5 and MA in P02 derives mainly from an imbalance of basic prognostic factors like percentage of soft tissue (L2.5: 27.5%; MA: 38.9%) or bone metastases (L2.5:38.2%; MA: 29.8%): in favor of MA. Finally, confirming the outcome of BC2, P02 should have more than1248 pts instead of just 602.

Conclusion: This is another example of two contradictory trials where randomized treatment assignment does not ensure comparability of the treatment groups to known prognostic factors. It would seem that if only moderate biases are in question, then all random errors and biases should be avoided. Despite the imbalance in favour of MA in P02, L2.5 is more potent than MA in various subgroups.

267 A randomized, placebo-controlled, explorative study to investigate the effect of low estrogen plasma levels on markers of bone turnover in healthy postmenopausal women during the 12-week treatment with exemestane or letrozole.

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Physiological bone loss begins after the age of 35 years and is enhanced primarily by estrogen deficiency (menopause) and aging. Anti-aromatase agents are approved for the treatment of postmenopausal, advanced, hormone responsive breast cancer and their efficacy is derived by their suppression of peripheral estrogen synthesis. Thus, one may expect a potential for increased bone loss in the presence of such agents. However, recent preclinical data indicate that in contrast to the non-steroidal, imidazole based aromatase inhibitors, the aromatase inactivator, exemestane, may not increase bone turnover because it is a steroid and has a weakly androgenic metabolite (17-hydro-exemestane). Both features might contribute to a protective effect on the bone in a hypoestrogenic condition.

The current study aims to investigate the effect of a 12-week treatment with exemestane 25 mg once daily po or letrozole 2.5 mg once daily po, on selected markers of bone turnover in healthy postmenopausal women, aged 50-75 years, with no prior history of osteoporosis. Further, a placebo control group to exemestane is utilized to compare the normal physiologic change in markers of bone resorption/formation that occur during the same 12-week time period. Total sample size is 60. Subjects are seen at baseline and at 2, 4, 8, 12 and 24 weeks, to perform clinical and laboratory safety tests and to obtain blood samples for assessment of estrogen plasma concentrations, bone specific alkaline phosphatases (BAP), C-terminal telopeptide of type I collagen (CTx), and urine samples for CTx and N-terminal telopeptide of type I collagen (NTx).

Enrollment is complete with sixty subjects randomized 1:1:1 to receive exemestane, letrozole or placebo to exemestane. Demographics include a median age of 60 years and all participants are of caucasian ethnicity. Primary endpoint is the change in bone turnover markers compared to baseline levels. Secondary endpoints include estrogen concentrations and safety. The study is being carried out at two Phase I units in Germany, Parexel GmbH and Pharm PlanNet Contract Research GmbH. Preliminary results will be presented.

268 Inhibition of intratumoral aromatase and estradiol by exemestane in postmenopausal breast cancer patients: results of a double blind randomized study.

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The effects of exemestane, a steroidal aromatase inactivator, on intratumoural aromatase activity and estradiol concentrations were investigated in a double blind, randomized, placebo controlled study. Twenty-six postmenopausal patients were treated, 16 with exemestane (25 mg once daily) and 10 with placebo (2:1 randomization), for 6-14 days before operation for primary breast cancer. In the exemestane treated group, sufficient amounts of tumour tissue were available from 9 patients for aromatase activity assay and from 13 patients for estradiol measurements. Sufficient amounts of fatty tissue in the exemestane group were available from 9 patients for both assays. In the placebo treated group, sufficient amounts of tumour and fatty tissue were obtained from 7 patients for both assays. Compared with the placebo group, aromatase activity was significantly lower in exemestane treated patients in both breast tumour tissue and breast fatty tissue (median reduction by 95% and 58%, respectively; P=0.0006 and P=0.04, resp.). Aromatase activity in fatty tissue was already very low at baseline (30 times lower than in tumor tissue), which may explain the lower reduction compared with tumor tissue. Estradiol concentrations were also significantly lower in the exemestane treated group in tumour, fatty tissue and plasma (median reduction by 81%, 32% and 94%, resp.; P=0.03, P=0.03 and P<0.0001, resp.). We conclude that orally administered exemestane effectively inhibits intratumoural aromatase activity and lowers estradiol concentrations in primary breast cancer. The inhibition of local aromatase activity may be an important mechanism for the anti-tumour activity of aromatase inhibitors.

269 Open-label, multi-center, controlled study of exemestane (E-Aromasin®) with or without celecoxib (Cx - Celebrex®) in postmenopausal women with advanced breast cancer (ABC) progressed on tamoxifen (T).

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Background: COX-2 is up regulated in 56% of breast tumors and tumor-derived PGE2 stimulates breast fat aromatase expression in ABC patients (pts). Paracrine loops may exist, activating PGE2-related aromatase or other growth-inducing pathways. E and Cx have anti-tumor activity in the female rats DMBA tumor model, and the ECx combination is synergistic. A randomized phase II study exploring the synergistic effect of Cx on E efficacy and tolerability in pts progressing on T has been initiated.

Material and Methods: Primary objective is Cx effect on E clinical benefit (OR+SD>=24 wks), secondary the effect of Cx on OR, OR duration, TTP, TTF and on E tolerability and pharmacodynamics. A 1-stage Fleming design is applied; the statistical hypothesis for clinical benefit (p0=0.35, pA=0.55) applies to ECx. The total sample size is 100 pts, randomized 1:1 with minimization for response to T, prior chemotherapy, and lesions site. Treatment is E 25 mg/po qd or E 25mg/po qd + Cx 400 mg/po bid. Pts with > 8 wks of T for ABC or adjuvant T for 6 (ER+) or 12 months (ER unknown) or PD within 12 months from adjuvant T, ECOG £ 2, measurable lesions and adequate bone marrow, liver and renal functions are eligible.

Results: To date 32 pts have been randomized with data available on 21, 11 E and 10 ECx. Median age (yrs): 66 E, 51 ECx, ECOG 0: 4 E, 5 ECx; Yrs from menopause: 8.1 E, 5.6 ECx; Prior hormone therapy only: 3 E, 3 ECx; ER +: 6 E, 8 ECx; Disease site: soft tissue 3 E, 2 ECx; soft + bone 3 E, 2 ECx; visceral 5 E, 6 ECx. Prior CHT for ABC: 5 E, 4 ECx; neoadjuvant /adjuvant CHT: 3 E, 3 ECx.

Discussion: To our knowledge, this is the first study exploring the effect of Cx on E anti-tumor activity and estrogen suppression, indirectly testing the role of PGE2 on aromatase activation in ABC pts. Results will be presented.

270 Prospective randomized celecoxib + anti-aromatase neoadjuvant phase II trial on postmenopausal hormone receptor positive primary breast cancer.

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Background: Anti-aromatase agents are active against advanced and metastatic breast cancer. COX-2 is over expressed in breast cancer and it is also a novel target of treatment. Celecoxib, a COX-2 inhibitor, has anti-angiogenic and apoptotic effects. Preclinical data has suggested that there may be synergy between exemestane and celecoxib in suppression of tumor growth. Animal studies have shown potent effects of exemestane in preventing bone loss. This Phase II study is a prospective randomised open-labeled trial which aims to examine the clinical and molecular responses of postmenopausal hormone receptor (+) primary breast cancer patients to different anti-aromatase regimens with or without celecoxib. Biological markers will be determined to evaluate the effects of these regimens in terms of tumor response, bone changes, and safety.

Materials and Methods: Ninety postmenopausal, receptor (+) women with histologically proven primary breast cancer are randomized to receive 3 months neoadjuvant therapy with exemestane (25 mg daily), exemestane + celecoxib (400 mg BD), or letrozole (2.5 mg daily). End-point assessments will be on response rate (clinical and pathologic responses), quality of life (Fact-b, version 4), angiogenesis (VEGF) and apoptosis (Caspase dependent cytokeratin degradation product M30) molecular markers, lipid profile serum and urinary bone turnover markers (bone alkaline phosphatase, C-terminal telopeptide, N-terminal telopeptide).

Results:

Initial results of the trial will be presented.

271 Preclinical studies evaluating the anti-tumor effects of exemestane using the intratumoral aromatase postmenopausal breast cancer model.

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The data reported to date suggest that aromatase inhibitors(AI) may become the preferred first-line therapy for postmenopausal patients with hormone-responsive breast cancer. In the present study, we have investigated the effects of the AI exemestane(Exe) on the growth of aromatase-transfected MCF-7Ca human breast cancer cells *in vitro* and *in vivo* . Aromatase activity assays were performed *in vitro* with MCF-7Ca cells in culture. Exe inhibited aromatase activity levels in MCF-7Ca cells with IC_{50} values of 1.37nM. The anti-tumor effects of Exe were determined and compared to the antiestrogen(AE) tamoxifen(Tam) using our intratumoral aromatase postmenopausal breast cancer model. MCF-7Ca cells were inoculated into female ovariectomized athymic nude mice and animals were supplemented with androstenedione Δ4A, 100µg/day) for the duration of the experiment. When the tumors reached a 300mm^3, the animals were assigned for treatment with either the Δ4A-vehicle, AI letrozole(Let) 10µg/day, Tam 500µg/day, or Exe 50, 100, 250, 500, and 1000µg/day. Tumor volumes were measured weekly. After 4 weeks of treatment the animals were sacrificed and the tumors and uteri were dissected. All of the treatments were effective at suppressing tumor growth. The volumes of the Tam-treated tumors increased by 0.35-fold, whereas tumors volumes in the Let-treated animals regressed by 15% over the 4 weeks of treatment. The inhibition of tumor growth by Exe was shown to be dose-dependent. At doses of 50 and 100µg/day tumor volumes increased by 2.1-fold and 0.9-fold respectively. In the animals treated with Exe at doses of 250, 500, and 1000µg/day, tumor volumes remained close to pretreatment levels indicating that these doses of Exe effectively inhibited tumor growth. Tumors weights in the control group (344±56 mg) were significantly greater than in all of the treatment groups (Let: 46±7 mg, Tam: 103±11mg, Exe50: 169±26mg, Exe100: 161±29mg, Exe250: 87±12mg, Exe500: 75±17mg, Exe1000: 84±15mg). Tam also significantly increased ($P < 0.001$) uterine weights compared to Let and Exe treatment. Our results suggest that the AI Exe is superior to AE Tam in controlling tumor growth and may be a more effective treatment for hormone-responsive breast cancer patients.

272 Exemestane as neoadjuvant treatment in patients > 65 years with T > 3 cm; preliminary results of a multicenter spanish phase II trial.

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Introduction: Aromatase inhibitors (AI) have shown activity in neoadjuvant setting higher than tamoxifen in patients with primary hormone-dependent breast cancer (BC). Exemestane is a highly active AI agent in metastatic BC.

Aim: To evaluate the efficacy of exemestane as neoadjuvant therapy as a primary endpoint. Secondary endpoints are rate of breast-conserving surgery, time to tumor progression, duration of response, and toxicity.

Material and Methods: 33 patients were enrolled with the following inclusion criteria: Histologic diagnosis of BC; no metastatic disease; T > 3 cm (T2, T3,T4a, T4b), Age >65; IK > 70%, ER (+) > 50 %, no previous hormone or chemotherapy treatment. Exclusion criteria were: Inflammatory BC, and premenopausal. At baseline all patients were considered not eligible for breast-conserving surgery. Patients were treated with exemestano 25 mg po/ d for 6 months. Tumor diameters were estimated clinically, by mammography and by breast ultrasound every 2 months.

Results: We present the results of 28 first evaluble patients. Patients characteristics were: Median age: 77 (68-87); stages included: T2-T3: 13, T4a-b: 15. Nodal status: N0: 17 , N1-2: 11, Grade: I:1, II:16, III:2 unknown: 9.

Radiological response using RECIST criteria:

Radiological R	Partial R	Stable D	Progression	Total
2 months	4 (14%)	24 (86%)	0	28
4 months	8 (36%)	14 (64%)	0	22
6 months	10 (50%)	9 (45%)	1 (5%)	20

Median time to surgery was 6.7 months; 15 patients have been operated on: 5 mastectomy and 10 conservative. The most frequent adverse events were: hot flushes, nausea, dizziness and pain.

Conclusion: These results shown an acceptable efficacy of the treatment and good tolerability in elderly population. Most of patients in response can conserve her breast. Further inclusion of patients and follow-up is needed.

273 Sensitizing effects of Aromasin on Paclitaxel action in human breast cancer cell lines.

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Background: Aromasin (exemestane) is a steroidal agent which causes inactivation of aromatase, the enzyme responsible for estrogen biosynthesis. There is preclinical evidence for the existence of a synergistic anti-tumour effect when Aromasin is combined with cytotoxic agents such as anthracyclines or taxanes. It is therefore expected that exemestane could be used to enhance the sensitivity of tumours for cytotoxic treatment in vivo. Complementary to actual clinical trials combining Aromasin and cytotoxic agents, we examined the effect of a combination of Aromasin and Paclitaxel (PTX) on growth of breast cancer cell lines. Material and Methods: In order to determine effects of Aromasin and Paclitaxel on growth of MCF-7 and MDA-MB-231 breast cancer cells, we used the colorimetric CellTiter MTS Assay (Promega). Cells cultured in serum-free medium containing 10µM androstenedione were treated with different concentrations of Aromasin and Paclitaxel (1 - 100 nM). Relative cell numbers of viable cells were determined after 24h and 96h of treatment. Results: We were able to demonstrate, that combined treatment with Aromasin and PTX is significantly more efficient than either of these substances alone. In ER-positive MCF-7 cells, 10 nM Aromasin did not reduce the number of viable cells after 4 days of treatment when compared to the negative control, but combination with 10 nM PTX (which alone reduced the cell number to 75.4%) resulted in a decrease of viable cells to 11.7% after 4 days. Weaker effects were observed in the ERα-negative breast cancer cell line MDA-MB-231. 4 days of treatment with 10 nM Aromasin resulted in reduction of viable cell numbers to 58.1%, 10 nM PTX alone decreased cell numbers to 62.7%, whereas a combination of both substances resulted in a reduction to 34.5%. Discussion: We were able to demonstrate a significant increase of cytotoxic Paclitaxel action triggered by Aromasin in MCF-7 and MDA-MB-231 breast cancer cells. Our data show a significant effect of Aromasin even in an ERα-negative cell line. This effect might be due to its inhibition of estradiol actions not being mediated by ERα. Our data suggest a clinical benefit of combined treatment with Aromasin and Paclitaxel, because the addition of Aromasin might allow a reduction of the Paclitaxel dose and therefore a reduction of its cytotoxic effects.

274 Intravenous administration of zoledronic acid offers long-term protection against bone loss in rats induced as a consequence of estrogen deprivation.

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Background: Aromatase inhibitors (AIs) are being evaluated in adjuvant endocrine therapy of hormone-dependent breast cancer. AIs induce estrogen-deprivation, a known consequence of which is increased bone loss. Bisphosphonates (BPs) have been shown to exert a bone protective effect in postmenopausal (PM) women (Reid et al. New Engl J Med 2002 346:653-661). We investigated whether bone loss induced in rats by either ovariectomy (OVX) or the AI letrozole (Let) could be prevented by the new potent BP zoledronic acid (Zol).

Material and Methods: Adult, skeletally mature, 8-month-old female Wistar rats were assigned to the following treatment groups (n=10): Sham (vehicle), OVX, Let-treated, OVX + Zol and Let + Zol. Zol was injected into the tail vein as a single dose of 0.8, 4 or 20 ug/kg either before OVX or before initiating daily oral dosing of Let (1 mg/kg) for 12 weeks. Changes in bone mineral density and structure were monitored non-invasively by peripheral quantitative computed tomography (pQCT) at 0, 2, 4, 8 and 12 weeks.

Results: Both OVX and Let resulted in the expected loss of bone, the initial loss due to OVX was higher than Let. OVX led to loss of 36 % cancellous bone and a 22% decrease in cortical thickness in the proximal tibial metaphysis whereas Let resulted in 18% and 19% decrease in these compartments respectively at 12 weeks. The effect of Zol was dose-dependent with 20 ug/kg fully protecting against both OVX- and Let-induced cancellous and endocortical bone loss at all time-points. At 4 ug/kg, the compound was fully protective for up to 8 weeks and significantly reduced cancellous bone loss at 12 weeks. Cortical thinning was also significantly reduced by 45% (OVX) and 64% (Let) respectively at the 4 ug/kg dose. The lowest Zol dose did not achieve statistically significant protection in any bone compartment at 12 weeks.

Discussion: Our data indicate for the first time that in rats, Zol dose-dependently protects against bone loss induced by daily oral Let, long-term effects of multiple dosing need to be determined. Zol, at a dose of 20 ug/kg, fully protected against Let-induced bone loss over the study period. These data support the use of Zol in preventing bone loss not only in otherwise healthy PM women but may also reduce bone loss in PM women undergoing adjuvant treatment with AIs in general and Let in particular. Direct dose extrapolation to humans can not be made.

275 Early assessment of apoptotic and proliferative response to tamoxifen does not predict for subsequent tumour response - a cautionary tale!

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There is increasing interest in the optimisation of treatment in early breast cancer by the use of predictive factors, with studies examining changes in treatment-related biological markers. We studied the sequential changes in apoptosis and proliferation in 30 post-menopausal women with locally advanced or large operable breast cancer who had been treated for 3 months' tamoxifen. Core biopsies were taken before therapy, after two weeks and at the time of definitive surgery.

Proliferation and apoptosis were assessed by Immunohisotchemistry (IHC), on a minimum of 3 000 invasive breast cancer cells, using the MIB-1 antibody and. the TUNEL assay respectively.

Medians		Apoptotic index			% MIB-1 +ve		
Day	n=	0	14	90	0	14	90
All tumours	30	0.25	0.53	0.32	9.0	11	9.3
Responders	22	0.25	0.55	0.32	7.5	4	4
Non-responders	8	0.23	0.4	0.23	9.0	15	14

Overall, between baseline and day 14, there was a trend for apoptosis to rise particularly in responding tumours ($p < 0.07$), and for mitosis to fall at ($p = 0.05$). Even when considering changes in the ratio of apoptosis: proliferation in individual tumours none reached statistical significance, although for responding tumours there was a trend for a rise ($p < 0.07$).

These data are consistent with a response after 3 months' tamoxifen being preceded by a rise in apoptosis and a fall in proliferation at 14 days, but the changes in individual tumours are not sufficient to direct therapy. This may be due to the variability seen when sampling the same tumour without any therapeutic intervention (Cameron et al SABC 2000, abstract 169).

276 Expression of mcl-1 predicts absence of local-regional recurrence of breast carcinoma.

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The aim of this retrospective study on archival tissue was to ascertain if dysregulated mechanisms of apoptosis and proliferation contribute significantly to tumor aggressiveness in the form of local-regional recurrence. The study investigated 145 cases of breast carcinoma, divided into two groups: (i) those with local-regional recurrence of tumor within a 5 year period following surgery (71 cases), defined as growth of tumor at the original site of surgery or in the axillary lymph nodes; (ii) those without local-regional recurrence during the same period (74 cases). The two groups were further subdivided into those with metastatic tumor in the axillary lymph nodes at presentation and those without axillary lymph node metastases at presentation. The expression of biological factors related to apoptosis and proliferation was detected by immunohistochemical staining of sections of archival paraffin embedded tissue. The degree of antigen expression was assessed semi-quantitatively by observing the intensity and frequency of cytoplasmic and nuclear staining using a combined graded scale. Nuclear and cytoplasmic expression of mcl1 was strongly correlated with absence of local-regional recurrence ($P < 0.001$). Investigations on pro- and anti-apoptotic factors showed no significant statistical relationships between local-regional recurrence and expression of the pro-apoptotic factors, Bax, Bak and p53 (immunonegative), the anti-apoptotic factors, Bcl2, Bcl-XL and Bag-1 and the proliferation factors, Ki67 and PCNA. Local-regional recurrence showed a near significant association with the rate of apoptosis as determined by the TUNEL method ($P < 0.06$). Tumor size (> 2 cm) showed a significant relationship to local-regional recurrence ($P < 0.03$). Bcl2 expression was associated with low tumor grade ($P < 0.01$) and immunoreactive p53 expression with high tumor grade ($P < 0.03$). Lymph node status at presentation was not related to local-regional recurrence. These results suggest that expression of mcl1 has a profound influence in reducing the risk of local-regional recurrence in breast carcinoma. This effect may be related to the known biological functions in promoting cell survival and cellular differentiation together with the control of cellular proliferation. The potential clinical role of mcl1 in controlling local-regional recurrence merits serious consideration.

277 Flavopiridol downregulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis.

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The molecular mechanisms underlying the cell-cycle and apoptotic effects of Flavopiridol (FP) (0.3 to 1.0 μM) were determined in the human breast cancer SKBR-3 and MB-468 cells. Treatment with FP caused accumulation in the G1 phase of the cell cycle and induced apoptosis. This was associated with downregulation of cyclins D1 and B1 levels, as well as inhibition of cdk1 and cdk2. FP-induced apoptosis was accompanied by a conformational change and mitochondrial localization of Bax, resulting in the accumulations of cytochrome c, Smac and Omi/HtrA2 in the cytosol, as well as PARP cleavage activity of caspase-3. Treatment with FP also attenuated the mRNA and protein levels of Mcl-1, Bcl-x_L, XIAP, cIAP-2 and survivin. As compared to the control, in the MB-468 cells with overexpression of Bcl-2 (468/Bcl-2), FP-induced Bax conformational change and apoptosis were significantly inhibited, while FP mediated decline in the levels of IAP proteins, Mcl-1and Bcl-x_L remained unaltered. The effects of co-treatment with FP and the non-taxane tubulin polymerizing agent Epothilone (Epo) B were also determined in MB-468 cells. A sequential treatment with Epo B followed by FP induced significantly more apoptosis of MB-468 cells than treatment with the reverse sequence of FP followed by Epo B or with each agent alone ($p<0.05$). Treatment with Epo B followed by FP was associated with more decline in the levels of XIAP, cIAP-2, survivin, cyclin D1 and Mcl-1. However, 468/Bcl-2 cells remained relatively resistant to Epo B followed by FP. Taken together, these findings suggest that increased apoptosis resulting from treatment of human breast cancer cells with Epo B followed by FP may be due to FP-induced downregulation of the antiapoptotic IAP, Bcl-x_L and Mcl-1 proteins. However, this treatment is unable to overcome the resistance to apoptosis conferred by Bcl-2 overexpression in breast cancer cells.

278 Role of proapoptotic Bim in the anti-breast cancer activity of taxotere or epothilone B.

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Bim is a BH3 domain only-containing proapoptotic member of the Bcl-2 family of proteins. Certain apoptotic stimuli induce Bim to translocate to mitochondria and neutralize the antiapoptotic function of Bcl-2, Bcl-x_L or Mcl-1. Bim levels are transcriptionally regulated and may set the threshold for apoptosis. Also, Bim loss is known to confer resistance to apoptosis due to treatment with taxol. For the present studies we have introduced a synthetic peptide of BH3 domain of Bim fused to the internalization domain of the antennapedia homeoprotein (Ant-BH3-Bim) into MB-468, and determined the sensitivity to taxotere or the non-taxane epothilone B (EPO906, provided by Novartis). Ant-BH3-Bim (25 to 50 μM) induced Bax conformational change and mitochondrial localization of Bax, inducing the release into the cytosol of death promoting cytochrome c, Smac/DIABLO and Omi/HtrA2. This triggered the PARP cleavage activity of caspase-3 and apoptosis. Co-treatment with taxotere or Epo B significantly increased Ant-BH3-Bim induced apoptosis of MCF-7 or MB-468 cells. We also stably transfected the cDNA of Bim by a doxycycline-inducible pSTAR vector into MB-468 (468/Bim) cells. Treatment with 2 μg/ml doxycycline significantly induced Bim protein expression and apoptosis of 468/Bim but not the control cells. In addition, while stable transfection and over-expression of Bim in MB-468/Bcl-2 (468/Bcl-2/Bim) cells did not affect survival, it sensitized these cells to taxotere or Epo B-induced apoptosis. Expression of all isoforms of Bim (extra-long, long and short) inversely correlated with the expression of Her-2 and its kinase activity in SKBR-3 versus MCF-7 cells, as well as in Her-2 overexpressing versus Her-2 negative tumor samples from patients. In addition, treatment with Herceptin significantly downregulated Bim expression in SKBR-3 cells. These studies indicate that Bim expression regulates the threshold for apoptosis and sensitizes breast cancer cells to antimicrotubule agents.

279 Active and protective immunity induced by a protein-based vaccine targeting the HER2/*neu* oncogenic protein.

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More than 185 000 new cases of breast cancer are diagnosed each year. Among them, 30% are shown to overexpress the HER2/ *neu* oncogene, which plays a crucial role in the pathogenesis and contributes to a poor clinical outcome. The HER2/ *neu* protein is an attractive therapeutic and immunogenic target. The existence of antibodies, helper T cells and cytotoxic T cell immunity to HER2/ *neu* has been demonstrated in patients with cancer. Moreover, passive transfer of HER2/ *neu* -specific monoclonal antibodies have been shown to be of clinical benefit in patients with HER2/ *neu* -overexpressing tumors. Inducing an active immune response including HER2/ *neu* -specific antibodies and T cells may result in long-lived immunity that may provide therapeutic benefit.

We generated a protein-based vaccine consisting of the purified HER2/*neu* protein formulated in a strong adjuvant. We show that mice developed both humoral and cellular responses to HER2/*neu* after several vaccinations. These Her2/ *neu* -specific immune responses were able to protect mice against a tumor challenge with a HER2/*neu* -expressing mouse tumor and implied both CD4 and CD8 T cells. We show that HER2/*neu* -specific antibodies could also be induced in rabbits and monkeys. The presence of functional antibodies that inhibit the *in vitro* growth of human breast cancer cells SKBR3 and the *in vivo* growth of human ovarian SKOV3 tumor xenograft has been demonstrated.

These studies show that a vaccine based on a purified dHER2 protein formulated in a strong adjuvant can induce a systemic anti-tumor immune defense, in which humoral as well as cellular responses are directed against both the extracellular and intracellular domains of the HER2/ *neu* oncogene.

280 Isolation and expansion of HER2-specific, tumor-reactive T cell clones from patients with HER2-overexpressing breast cancer: prospects for adoptive T cell therapy.

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Background: Attempts to eradicate cancer by adoptive T cell transfer have been limited due to the difficulty of generating T cells with defined antigen specificity. The current study focuses on the generation of cytotoxic T lymphocytes (CTLs) against the human epidermal growth factor receptor 2 (HER2) being overexpressed by 20-30% of breast cancer.

Material and Methods: Dendritic cells (DCs) were generated from peripheral blood monocytes and pulsed with the HER2-derived peptide 369-377 (HER2p369) known to be naturally processed and presented with HLA-A2. HER2p369-pulsed DCs were used for stimulating autologous peripheral blood lymphocytes from HLA-A2 positive healthy donors and breast cancer patients. Following 3-4 weekly stimulations, HER2-specific T cells were visualized by fluorochrome-labeled HLA-A2/HER2p369 multimers. HER2-specific T cells were sorted, cloned and further expanded in vitro.

Results: Baseline frequencies of CD8+ HER2p369-reactive T cells were less than 0,01% of all PBMCs as determined by multimer staining. After several stimulations the frequency of HER2p369-specific T cells increased up to 0,04% - 0,2% of PBMCs. Multimer-positive T cells were sorted, cloned and further expanded. The calculated yield after 3 rounds of expansions was more than 10e10 cells per T cell clone. The expanded CTL clones lysed HER2p369-pulsed target cells and HLA-A2 positive, HER2-overexpressing tumor cells. Using multimer-guided sorting, tumor-reactive CTLs against HER2p396 could be isolated from healthy donors and from patients with advanced breast cancer. Our current work focuses on the isolation and cloning of CD8+ CTL and CD4+ T helper cells against additional HER2-derived epitopes presented by HLA class I and II molecules.

Discussion: The use of fluorochrome-labeled HLA/peptide-multimers permits the detection and sorting of HER2-specific T cells present at low frequencies. The ex vivo expansion protocol yields numbers of HER2-reactive T cell clones sufficient for adoptive T cell therapy. Ongoing clinical studies focus on the adoptive transfer of autologous HER2-specific T cell clones in patients with HER2-overexpressing breast cancer.

281 The short-term safety and immune responses of HER-2 DNA AutoVac™ (ME 103.1.1) in patients with HER-2 positive metastatic breast cancer. Results from the first dose level of the Phase I multi-dose trial.

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Background: HER-2 DNA AutoVac™ (ME 103.1.1, Pharmexa A/S, Denmark) is a therapeutic vaccine targeting HER-2 overexpressing tumours. HER-2 DNA AutoVac™ encodes a modified HER-2 antigen including two highly immunogenic peptides derived from tetanus toxin. In animal models the vaccine gave rise to HER-2 specific cytotoxic T lymphocytes (CTL) and polyclonal antibodies (Ab) resulting in significant tumour inhibition.

Objective: To evaluate short-term safety of i.m. HER-2 DNA AutoVac™ in patients with metastatic breast cancer and to evaluate the ability of the vaccine to break tolerance for the self-protein HER-2 by raising cellular and/or humoral immune responses.

Materials and Methods: 9 women (age: 42-62 yrs) with HER-2 positive breast cancer (IHC +2 (n=1); +3 (n=8), DAKO HercepTest) were enrolled to the lower dose level of an open label, multicentre, dose escalation trial. HER-2 DNA AutoVac™ (0.2 mg naked plasmid DNA) was injected on day 1, 15, 29, 43 and 57 followed by 16-weeks follow-up period. Patients who had at least one vaccination were included in the safety population. Adverse events were graded according to the NCI common toxicity criteria. HER-2 specific T-cell responses were analysed by IFN-gamma ELISPOT; Ab responses by ELISA.

Results: Possibly related adverse events (AEs, n=2) were reported in two patients: pruritus (grade 1) and pain in hip (grade 2); the events had no impact on study treatment and the patients recovered without sequelae. Serious AEs (n=7) reported in 4 patients were not related to study drug. Preliminary immunological data demonstrated HER-2 specific T cell responses in 2 of 5 evaluable patients. In 4 patients HER-2 specific IgG Ab were detected (3 had Ab present before vaccination). T-cell reactivity coincided with the presence of HER-2 antibodies.

Conclusion: Active immune-therapy with up to five repeated doses of 0.2 mg HER-2 DNA AutoVac™ is safe and, even at the lowest dose level investigated, effective at activating HER-2 specific T cells and raising antibodies in patients with HER-2 positive metastatic breast cancer. Additional data will be presented at the congress.

282 Targeted delivery of radioiodinated anti-EGFR MAb 425 in human breast carcinoma cells: influence of the chemotherapeutic taxol.

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Background: Epidermal growth factor receptor (EGFR) over-expression has been documented in brain and breast carcinomas, and is indicative of a poorer prognostic outcome. I-125 MAb 425 is currently being used as an adjuvant treatment for human high grade gliomas in a Phase II clinical trial and shows increased survival. This therapy is being investigated in breast cancer cells.

Material\Methods: Using human breast carcinoma cell lines of varying EGFR expression, 200,000 cells were incubated for 24-96 hours in tissue culture plates with taxol concentrations ranging from 10 uM to 0.01 nM. Treated L15 culture media was removed at designated time periods and aherent cells were subsequently incubated with a saturating concentration (37 kBq) of I-125 MAb 425. Supernatant, receptor-bound, and internalized cellular contents were collected and assayed for radioactive uptake using gamma scintillation.

Results: Pretreatment of the human breast carcinoma cell lines with taxol affected EGFR binding and internalized cellular acccumulation of I-125 MAb 425 in a time- and concentration-dependent manner. Nanomolar concentrations enhanced EGFR binding of radioiodinated MAb 425 starting at 48 hours. Compared to cells incubated with I-125 MAb 425 only, receptor-bound radioactivity for taxol-treated cells was 28% greater for BT-20 using 10 nM at 72 hrs pretreatment, 37% more for MB-468 using 1 nM at 72 hrs, and 94% enhanced for MB-231 using only 0.1 nM at 48 hrs pretreatment. Taxol-treated BT-20 cells showed the greatest increase (26%) in cellular accumulation of I-125 MAb 425 with 96 hrs preincubation. Cellular uptake was greatest for MB-468 and BT-20 cells with EGFR overexpression, accumulating 8- and 12-fold more activity respectively, than MB-231.

Discussion: Since earlier studies have demonstrated that I-125 MAb 425 has a similar pharmacokinetic profile for both high grade gliomas and breast carcinomas with EGFR overexpression, I-125 MAb 425 may be beneficial as an adjuvant treatment for breast cancer patients. With the established impact of chemotherapy on cancer patients, we believed it prudent to investigate the effects of taxol in relationship to a targeted clinical radioimmunotherapeutic approach to evaluate possible synergism.

283 Intracellular cytokine profile of T cells from women with breast cancer: immune dysfunction and micrometastases.

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Background: Cytokines produced by T helper (Th) and T cytotoxic (Tc) cells are critical to the efficacy of a given immune response. Type 1 cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor α (TNF-α) tend to drive cell-mediated immune responses while type 2 cytokines such as IL-4, IL-6, and IL-10 tend to drive humoral immune responses. Dysregulation of the normal balance of type 1 and type 2 T cells may result in impaired cell-mediated immunity in cancer patients. In this study, we assessed the intracellular cytokine profiles of both CD4+ and CD8+ T cells in the peripheral blood of women with breast cancer. **Materials and Methods:** We analyzed peripheral blood samples from patients prior to their first surgical resection and 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-γ, TNF-α, and IL-4). Bone marrow samples from some of these patients were also collected and analyzed for the presence of epithelial cells (micrometastases) by flow cytometry. **Results:** The percentages of both CD4 and CD8 cells producing type 1 cytokines (IL-2, IFN-γ or TNF-α) were significantly lower in patients with breast cancer compared to healthy controls. Similarly, IL-4 producing CD4 and CD8 T cells were significantly decreased, suggesting a general immune dysfunction in these patients as opposed to a shift in the balance of type 1 and type 2 cells. These dysregulated T cell responses did not correlate with stage of disease nor with nodal status. However, we did observe a correlation between numbers of micrometastases in the bone marrow and T cell responsiveness. For example, patients with the lowest IL-2 or IFN-γ responses tended to have the highest numbers of micrometastases whereas patients with higher percentages of IL-2 or IFN-γ producing T cells tended to have fewer micrometastases. **Discussion:** Our results demonstrate a general immune dysfunction in both CD4+ and CD8+ T cells in women with breast cancer (prior to any therapy). A depression in both type 1 and type 2 cytokines was observed. In addition, this immune dysfunction correlated with the presence of circulating micrometastases.

284 Reversal of defective dendritic cell function in vitro in women with operable breast cancer: important role of interferon-α.

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Background:

Escape from immune surveillance is a fundamental biological feature of malignant disease in man, contributing to uncontrolled tumour growth. Dendritic cells (DCs) play a crucial role in initiating and developing cell-mediated immune responses. Defective DC function has been demonstrated in malignant disease. Reversal of this dysfunction is a prerequisite for the induction of effective anti-cancer immune responses.

Methods:

DCs were generated from the peripheral blood (PB) and lymph nodes (LNs) of women with operable breast cancer by culturing monocytes in cytokine-conditioned medium (CCM). Monocytes were isolated, using density gradient centrifugation and plastic adherence, and cultured in vitro, supplemented with recombinant human (rh) granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-4 (IL-4), tumour necrosis factor-α (TNF-α) and interferon-α (IFN-α). The stimulatory capacity of DCs in the allogeneic mixed leukocyte reaction (MLR), macropinocytosis of dextran particles, expression of surface activation and maturation markers and production of cytokines were determined.

Results:

PBDCs and LNDCs from CCM demonstrated a significantly higher capacity to stimulate in an MLR ($p<0.01$) and expressed significantly higher levels of HLA-DR, CD40 and CD86 ($p<0.01$), compared with freshly isolated PBDCs and LNDCs from patients with breast cancer. Also, CCM generated PBDCs showed a significantly enhanced macropinocytotic capability ($p<0.01$) and IL-12 secretion in vitro ($p<0.01$). Optimal DC activation was seen in CCM containing IFN-α.

Conclusion:

Our study shows that dysfunctional DCs, isolated from patients with breast cancer, can be reversed by in vitro culture with cytokines and highlights the importance of IFN-α.

285 The effect of aging on the progression of breast tumors and T cell responses in vivo.

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Background: Some tumors in the elderly are biologically less aggressive in both animal tumor models and in humans. This might be a combination of different phenomena such as cellular senescence, the level of expression of growth receptors and tumor-associated (TAA) at the cell membrane of tumors. However, an age-related decline in T cell function should favor tumor growth in the elderly. To obtain a better understanding of the potential role of age-related decline in T cell immunity in the growth of breast tumors in the elderly, we studied the effect of aging on the progression of breast tumors and on CD4+ and CD8+ T cell responses in vivo.

Materials and Methods: Various numbers of cells of mouse mammary tumor cell line 64pT (103, 104, or 105) were injected into the mammary fat pads of immune competent normal Balb/C mice of 3, 9 or 24 months. Sixty nine, 49, or 29 days later the mice were sacrificed and analyzed for the following parameters; tumor size, tumor weight, onset, frequency as well as CD4+ and CD8+T cell responses in the draining lymph nodes (inguinal) and in the spleen. This study was repeated with another breast tumor cell line 4TO7 (104 cells only).

Results: A trend of more aggressive breast tumors in the mice of 3 months than in the 9 or 24 months old was observed in the group that received 104 tumor cells only. There was no significant difference in onset or frequency of the breast tumors. A stronger vascularization of the 4TO7-induced tumors was observed in 3 than in 9 or 21 months old mice. Metastases in the lungs were observed only in mice of 21 months injected with 4TO7. Metastases in the peritoneal cavity were predominantly found in the older mice injected with 64pT or 4TO7. A significant increase in the percentage of CD8+ T cells was observed in the draining lymph nodes of mice of 24 months compared to mice of 3 or 9 months old (P=0.0054). These CD8+ T cells will be cloned and analyzed for receptors involved in T cell activation. In addition, the tumor cells will be analyzed for antigens involved in T cell activation and for receptors involved in growth of breast tumor cells.

Discussion: In conclusion, obtained results showed a trend of more aggressive tumors in young than in old mice. The effect of aging on T cell responses and its potential role on tumor progression will be discussed.

286 Activation of the erythropoietin-receptor pathway does not affect chemo- or radiosensitivity of EpoR expressing cancer cells

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Background: Anemia in cancer patients is associated with reduced quality of life and local failure after radiation treatment. Therefore, erythropoietin (Epo) is now widely used to correct tumor- or chemotherapy-related anemia. Erythropoietin-receptor (EpoR) signaling mainly acts via activation of STAT5, but also cross-activates the anti-apoptotic transcription factor NF-κB. This causes neuro-protection against oxidative stress and may imply possible chemo- and radioprotection of cancer cells. **Material&Methods:** In order to investigate possible protective effects of EpoR signaling we used an *in-vitro* model system employing HeLa TetOff cells, stably transfected with an expression vector for the EpoR gene. Activation of the anti-apoptotic transcription factor NF-κB was assayed using EMSAs. Protein levels of the inhibitor IκB, EpoR and phosphorylated forms of STAT 5 were visualized by western blotting. Alteration of radiation sensitivity was monitored using clonogenic assays. Sensitivity to chemotherapeutic drug was analyzed by dye exclusion tests. **Results:** Expression of EpoR resulted in tyrosine phosphorylation of STAT5. Using EMSAs we could demonstrate strong activation of NF-κB by EpoR signaling. Activation of NF-κB did not require degradation of IκBα and was not prevented by proteasome inhibition. Although stimulation with Epo resulted in cytoprotection of EpoR-expressing cells, it did not alter intrinsic radiosensitivity or sensitivity to cis-platin, cyclophosphamide or doxorubicin. **Discussion:** Our results underline the safety of anemia correction by Epo in anemic patients undergoing radiation therapy and/or chemotherapy, even in cases where tumor cells express the erythropoietin receptor.

287 Comprehensive gene expression analysis predicts postoperative prognosis of ER-negative breast cancer.

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Background: ER status in breast cancer is critical since ER-negative breast cancer patients often have compromised survival rates and systemic chemotherapy. To find novel genetic prognostic markers in ER-negative breast cancer, here we analyzed gene expression profiling by cDNA microarray in more generalized ER-negative breast cancer between 5 years survivors (5Y-S) and those dead within 5 years (5Y-D) after primary operation.

Materials and Methods: We selected ER-negative breast cancers (invasive ductal carcinoma). The samples were divided into 5Y-S and 5Y-D groups. Total RNA was extracted from normal and cancer tissue under strict quality control, then amplified with a T7-based method and labeled with Cy3 or Cy5. The competitive hybridization was performed with 25344 genes on in-house-spotted glass slides. The procedure was performed with an automated slide processing system. Data normalization was done using 32 housekeeping genes as an internal control. Array results were confirmed by RT-PCR.

Results and Discussion: Several genes and ESTs were up-regulated significantly in each group with few overlapping. The gene expression profiling was quite different between 5Y-S and 5Y-D. The characteristic expressed genes in each group may serve as new genetic prognostic markers.

288 The progression of DCIS to IBC: a cDNA expression microarray study.

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BACKGROUND: Ductal carcinoma in situ (DCIS) is very common. It is important because most invasive breast cancers (IBCs) arise from DCIS. Additional genetic defects must occur in DCIS to cause its progression to IBC. Knowledge of these defects could be useful clinically as prognostic factors to assess the risk of progression and, more importantly, as therapeutic targets to prevent progression. STUDY DESIGN/METHODS: Frozen samples of pure DCIS (n = 23) and pure IBC (n = 19) were evaluated using cDNA expression microarrays (Affymetrix HG-U95Av2 Chip containing 12,000 annotated genes). The samples were not microdissected to retain potentially important genes from non-tumor cells (e.g. in the stroma) but they were screened to contain a high level of tumor cellularity (>75%). Data was evaluated and compared using dChip software. RESULTS: Following normalization of results between arrays, the data was filtered to eliminate consideration of genes with very low expression, which resulted in 978 genes from the original 12,000 on the chip. In preliminary analyses, an unsupervised hierarchical clustering of these 978 genes (i.e. an unbiased sorting of the genes and samples) resulted in perfect separation of DCIS and IBC. A supervised comparison of these results (i.e. a directed look at specified groups of samples and genes to illustrate the most discriminating genes expressed at >3-fold differences between DCIS and IBC at a p-value of <.01) resulted in 148 genes. The pathways these 148 genes are implicated in were diverse but dominated by genes involved in cell adhesion, extracellular matrix, motility, and differentiation - all reasonable categories for invasion-related biology. The arrays results of interesting genes are being confirmed at the RNA level by RT-PCR and evaluated for expression profiles and clinical significance at the protein level by IHC on tissue microarrays of human DCIS and IBC - the presentation will show results from these ongoing studies. CONCLUSIONS: A large number of genes have been identified by cDNA expression microarrays which may be important in the progression of DCIS to IBC.

301 A transforming growth factor beta 1 polymorphism is protective against breast cancer.

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Transforming Growth Factor Beta (TGF-b) is the prototype of a large family of paracrine factors, which play a crucial role in growth inhibition. A TGFB1 polymorphism has been associated with increased serum TGFB1 levels and decreased incidence of breast cancer. Moreover, a type I TGF-β receptor (TGFBR1) polymorphism coding for a hypomorphic receptor has been associated with an increased incidence of both colon and breast cancer. TGFB1 and TGFBR1 polymorphisms affect TGF-b signaling levels independently. Here we present data from a case-control study on one polymorphism of the TGFB1 gene.

DNA from peripheral blood samples of 213 cases with breast cancer and 213 healthy controls, matched one-to-one for gender and ethnic background, were analyzed. One TGFB1 polymorphism (T10→C10) was detected by a PCR based assay, which resulted in an arginine to proline substitution.

We found that 16.4% of our cases were C10/C10 carriers compared to 26.8% of the controls (p=0.017, McNemar's non-parametric test) (table 1). The T10/T10 genotype was found in 31.5% of our cases and in 29.1% of our controls and the T10/C10 genotype in 52.1% of our cases and 44.1% of our controls. Analysis of the data for the two main ethnic groups (Caucasians and African-Americans) showed that the frequency of the C10/C10 genotype differs in the two groups. We found that 28.6% African-American breast cancer cases and 17.9% carried the C10/C10 genotype. Among Caucasian cases and controls the C10/C10 frequency was 14.7% and 28.2% respectively.

The C10/C10 genotype has been shown to be protective against breast cancer in elderly white females. We are presenting similar findings in a multiethnic population of breast cancer patients with a one-to-one match. However, there is a discrepancy in the frequency of the C10/C10 genotype within different ethnic groups. These results warrant validation studies in various tumor types as well as different ethnic groups to determine the association of TGFB1 and TGFBR1 polymorphisms with cancer risk.

Table 1. TGFB1 polymorphism frequency among cases and controls.

	T10/T10 n (%)	T10/C10 n (%)	C10/C10 n (%)
Controls (n= 213)	62 (29.1)	94 (44.1)	57 (26.8)
Breast Cancer Patients (n=213)	67 (31.5)	111 (52.1)	35 (16.4)*

* p=0.017 (McNemar's Test)

302 Geographic variation in incidence correlates with risk for developing estrogen and progesterone receptor (ER, PR) positive breast cancer.

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Geographic variations in breast cancer incidence have long been noted. In particular, rates of invasive disease in affluent Marin county are 25% higher than those in comparable non-Hispanic White females living in other San Francisco Bay Area and urban California counties. Surprisingly, this Bay Area geographic difference in breast cancer incidence occurs primarily in women aged 50-70. Since the proportion of receptor (ER, PR)-positive breast cancers arising after age 50 may be linked to race/ethnic and geographic differences in incidence and associated with increased reproductive (R) and socioeconomic (SE) risk factors, we used data from the Surveillance, Epidemiology, and End Results (SEER) program to identify ER and PR tumor status (-,+) for all breast cancer cases arising between 1992-1998 in Marin (M) vs. four other Bay Area counties (BA: Alameda, Contra Costa, San Francisco, San Mateo). The number of cases and mean proportion (with 95% confidence intervals) of breast cancer subtypes (ER+/PR+, ER+/PR-, ER-/PR+, ER-/PR-) were compared according to age at diagnosis and by race/ethnic group. For all breast cancer subtypes before age 50 and after age 70, and for the two ER+/PR- and ER-/PR+ subtypes arising at any age, there were no significant differences between M and BA breast cancer cases. In contrast, M women between ages 50-70 had proportionately more ER+/PR+ tumors (up to 75%, vs. 65-69% for BA) and proportionately fewer ER-/PR- tumors (down to 6%, vs. 15-19% for BA). Thus, differences in M vs. BA breast cancer incidence rates since 1992 are consistent with the enrichment of a subpopulation of M women (aged 50-70) for R and SE risk factors predisposing toward development of receptor-positive breast cancer.

303 Inflammatory breast carcinoma and non-inflammatory locally advanced carcinoma of the breast, distinct clinicopathologic entities?

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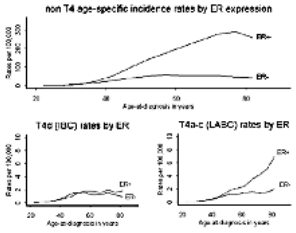
Background: Although both inflammatory breast carcinoma (IBC) and non-inflammatory locally advanced breast carcinoma (LABC) are associated with poor prognosis, whether they are distinct clinicopathologic entities is controversial.

Objective: To determine if IBC and LABC were different, we compared incident breast cancer characteristics in the Surveillance, Epidemiology, and End Results (SEER) Database.

Methods: SEER extent of disease codes identified IBC and LABC, corresponding to the American Joint Committee on Cancer clinicopathologic designation TNM T4d and T4a-c, respectively.

Results: IBC (n=2,237) versus LABC (n=3,140) was associated with younger mean age-at-diagnosis (58.4 versus 66.3 years), positive axillary nodal status, poor histologic grade, and negative estrogen receptor expression (p<0.0001). IBC age-specific incidence rates (or risks) increased premenopausally (<50 years) then stabilized to a constant rate, whereas LABC risks increased continuously with advancing age. We also stratified IBC and LABC risks by ER expression. As previously described for sporadic (non-familial) breast cancer, LABC showed discordant-dichotomous ER rates, i.e., ER+ rates increased with aging while ER- rates flattened after menopause (>50 years). On the other hand, IBC showed a novel concordant pattern where both ER+ and ER- rates rose premenopausally then flattened after menopause.

Conclusion: IBC and LABC appeared to be distinct clinicopathologic entities as evidence by early age-at-onset, poor prognostic factor profile, and age-specific risk pattern. Concordant IBC ER risk was a unique observation, which has not been previously described. Failure to increase after menopause possibly suggested that premenopausal reproductive hormones had a greater impact upon maintaining IBC than LABC risks.



304 Application of the lognormal model for prediction of long-term survival rates from short-term follow-up data in advanced breast cancer.

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Background: This study tests the use of a parametric lognormal model for predicting long-term survival rates of patients treated with modern treatments including surgery, chemotherapy, radiotherapy, and hormonal therapy.

Materials and Methods: From 1981- 1995, our provincial cancer registry had 5894 cases of breast cancer. Phase 1 used the minimum chi-square test to assess the goodness of fit of the survival time to a lognormal distribution among those patients who died with their disease present. Phase 2 used the maximum likelihood method to estimate long-term survival rates using short-term follow-up data. To validate the lognormal model, the estimated long-term cause-specific survival (CSS) rate was compared with the calculated values by the Kaplan-Meier (KM) actuarial methods using long-term data.

Results: The survival time of the uncured group of stage III and IV followed a lognormal distribution. An empirical formula was developed to bridge phase 1 and 2 for choosing the optimum period of cohort and follow-up duration required for phase 2. For stage III, a 3-year period was suggested by the formula and patients were followed-up as a cohort for an additional 6 years; e.g., from a cohort of 174 patients predicted the 15-year CSS to be 12% by lognormal model. The KM calculation using actual follow-up data for 15 years was 14%. For stage IV, a 2-year cohort was used and patients were followed-up for an additional 6 years: a cohort of 135 patients estimated 15-year CSS to be 1% (lognormal prediction) and 2% (KM calculation).

Discussion: The lognormal model was validated for the prediction of long-term survival rate of advanced breast cancer, in addition to other sites in the literature. The practical value is that this lognormal model could predict the results of prospective trials earlier. Advances in cancer treatment might be evolving faster, resulting in a reduction of cost in cancer research.

305 The inflammatory breast cancer registry: an approach to standardization.

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Background: Inflammatory breast cancer (IBC) is a rare highly aggressive form of cancer, which seems to disproportionately affect black women. Although IBC is recognized as a specific clinical entity, diagnostic criteria for IBC are controversial. The purpose of the IBC Registry (IBCR) is to develop a large, centralized and standardized resource of IBC cases that could be used to refine diagnostic criteria and characterize the epidemiological, clinical, pathological and molecular characteristics of these tumors.

Methods:The IBCR is recruiting all patients suspected of having IBC who consent to participating in an interview assessing risk factors and whose tissue blocks are available for laboratory evaluation. Initially, patients are classified according to clinico-pathologic criteria into three groups: (1) clinical presentation typical of IBC with pathologic confirmation; (2) clinical presentation typical of IBC without pathologic confirmation; (3) pathologically defined IBC without typical clinical features. Subgroups will include patients with incomplete criteria according to AJCC definition, e.g. redness, warmth and edema involving less than half the breast, edema (peau d'orange) without redness, etc.

Results: Thus far, we have studied IBC patients in Tunisia, California and the George Washington University Medical Center to establish our data collection system. A preliminary study comparing 45 IBC cases to 22 non-IBC breast cancer controls from Tunisia has recently suggested that IBC is associated with increased microvessel density (McCarthy et al, ASCO, 2001). Additional ongoing work is focusing on whether mouse mammary tumor virus sequences are associated with IBC (Coronel et al, submitted).

Conclusion: The centralized collection of specimens and data in the IBC registry will be made available to investigators throughout the breast cancer research community. It is hoped that this project will lead to molecular characterization of IBC and a more objective classification of IBC patients.

306 Prevalence of anemia in Medicare-eligible breast cancer patients treated by chemotherapy or radiotherapy.

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Anemia has been suggested to be a common complication in breast cancer treated by chemotherapy and/or radiotherapy due to suppression of proliferative activity or stem cell ablation in the bone marrow. We evaluated the prevalence of anemia in a Medicare-eligible 1998 cohort of breast cancer patients treated with parenteral chemotherapy, radiotherapy, both, or neither. The 5% sample of Medicare Part A and B claims contains medical services information, which were screened to determine the diagnosis of breast cancer, metastatic activity (axillary, systemic, or bone), and of anemia, using ICD-9CM diagnosis and procedure codes and therapeutic modality by HCPCS codes. By definition, a code for anemia should occur twice during the year to confirm that diagnosis.

There were 22,203 patients (1.78% of total) with a breast cancer diagnosis in the 5% sample, of whom 967 (4.4%) received parenteral chemotherapy only, 1,939 (8.7%) received radiotherapy only, 855 (3.9%) treated with both, and the remaining 83.5% received neither during the cohort year. The prevalence of anemia was 34.1% in patients treated with parenteral chemotherapy, 12.7% in patients treated with radiotherapy, 32.4% in patients treated with both, and 12.8% in patients who did not receive either treatment. The prevalence of anemia by metastatic state and therapeutic modality is presented in the table below:

	No Reported Metastasis	Axillary Lymph Nodes	Systemic Metastasis	Bone Metastasis
Chemotherapy	34.1%	33.7%	37.9%	35.7%
Radiotherapy	12.7%	19.2%	21.5%	27.1%
Chemo+Radio	32.4%	29.6%	37.1%	44.1%
None	12.8%	15.0%	17.0%	18.5%
Overall	14.5%	22.5%	24.6%	28.7%

We conclude that the prevalence of anemia in breast cancer patients increases with disease severity, and is most predominant in patients receiving chemotherapy. Radiotherapy does not appear to impact any increase in anemia prevalence in these patients except when bone metastasis occurs.

307 Increasing incidence of invasive lobular breast cancer in the Swiss Canton of Geneva.

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Background: Recent publications have suggested a relation between the use of hormone replacement therapy (HRT) and the development of invasive lobular breast cancer (ILC). As the use of HRT in the Swiss canton of Geneva is rapidly increasing, we set out to study the incidence trends of ILC.

Methods: All incident cases of invasive breast cancers (n=6247) recorded between 1976-1999 at the Geneva Cancer Registry were included and classified into three categories: ductal carcinoma, lobular carcinoma and other carcinomas. Age adjusted incidence trends (European age standardised) were estimated by means of log linear regression analysis based on the maximum likelihood method. The effect of HRT was estimated by means of age-period-cohort analysis, hypothesising that HRT was introduced after 1975, in full use after 1995 and mainly used by women between 50 and 69 years.

Results: The incidence of ductal carcinoma increased 1.2% per year ($p<0.001$) from 85.2 to 110.1 / 100.000. This increase was limited to women aged 50-69 years and started after the introduction of mammography screening in routine health care. The incidence of other histological subtypes decreased 1.3% per year ($p<0.05$) from 28.2 to 25.5 / 100.000 and was due to a decrease of carcinoma not otherwise specified and without histological or cytological confirmation among the elderly (>80 years). The incidence of lobular carcinoma increased 14.4% per year ($p<0.01$) from 2.9 to 20.5 / 100.000. Age-period-cohort analyses demonstrated strong, significant differences in ILC incidence according to age and date of birth. The increase of ILC incidence was limited to women aged 50-69 born after 1925. A cohort effect was not present for women below the age of 50 or after the age of 70.

Conclusion: Over the past three decades, there was a disproportionate increase of invasive lobular breast cancer in the Swiss canton of Geneva. This study provides some clues that increasing use of HRT may have played a role in this increase. Additional research on ILC incidence according to use of HRT is currently carried out and results will be available in december 2002.

308 A French National Representative Survey of Early Breast Cancer Management. First results on hormone receptors.

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Background : The steady increase of breast cancer (BC) incidence and the availability of several new drugs have prompted a national representative survey of early infiltrating BC management. We present here the first results detailing the assessment of hormone receptors status (HR).

Patients and Methods : From Sept. 2001 to Apr. 2002, investigators from 124 Oncology and Surgery Departments covering all French Metropolitan Territory have prospectively collected data on 1295 consecutive patients (pts) with newly diagnosed invasive BC. 136 pts were non eligible. All data describing epidemiological features, surgical procedures, pathological diagnosis, assessment of HR status, radiation therapy, chemotherapy, and hormonal therapy were collected.

First Results : Median age was 57 years (26-100). 762 pts were menopausal (65.7%), of whom 289 had received hormone replacement therapy (37.9%), and 286 had BC familial history (25.8%). Most pts had low stage BC: stage I 46%; stage II 42.5%; Stage III 8.1%. Only 40 pts (3.5%) had stage IV disease. Invasive ductal carcinoma was present in 982 pts (84.7%), and lobular invasive carcinoma in 170 pts (14.9%). 572/1047 assessable pts were node negative (54.6%). SBR grading was available for 1093 pts. Grade I 23.5%; grade II 45.1%; grade III 27.8%. Assessment of HR statut was available for 1146 pts (98.8%). Results are detailed in the table (Enzyme Immuno Assay, EIA; Radio Immuno Assay, RIA; ImmunoHistoChemistry, IHC).

Conclusion : We present here a survey thoroughly representing the different French Oncology/Surgery departments and the population of pts with invasive BC. Very interestingly, almost all pts had a determination of HR status, contrasting with the high proportion of pts with "unknown" status in prospective trials. IHC has become the method of choice in a short span of time and certainly deserves a prospective evaluation. A detailed description of therapeutic strategies will be provided.

	HR Status		
	EIA	RIA	IHC
ER	82.7% (43/52)	87.5% (28/32)	80.7% (835/1035)
PR	79.2% (38/48)	68.7% (22/32)	70.6% (703/995)

309 Ten-year survival in a population-based breast cancer cohort from Goiania, Brazil, 1988-90.

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Background. Breast cancer shows the highest incident cause of cancer among women in Brazil (45.0/100.000 women), being also an important cause of death. Few breast cancer hospital-based studies have been carried out in the country, but any at a population-based set.

Material and Methods. All incident breast carcinoma cases (308 women with histopathologic confirmation)diagnosed in the city of Goiania between 01/01/1988 and 31/12/1990 were traced towards the local population based cancer registry. A ten-year follow-up was carried out following diagnosis until 12/31/2000, when each cohort member vital status (dead or alive) was ascertained by consulting cancer registry files, national mortality system, national health insurance files and voting registers.

Overall and relative (age adjusted for other causes of death) survival were obtained according to age, marital status, race, education and tumor morphology. Further, Kaplan-Meyer curves were obtained and the respective curves compared by the log rank test.

Results: Overall and relative breast cancer survival were 90 and 98% in the first year after diagnosis, 57 and 64% in 5 years, and 42 and 48% in ten years. Highest ten-year survival was observed for 65-74 yr. old women (49%), white (44%), high education level (50%), single (66%) and with low grade ductal carcinoma (71%). The poorest survival results were seen among 75 yr. and older women (18%), non-white (38%), low education level (42%), married, divorced and widowed (38%, p = 0.02) and with ductal infiltrating carcinoma (39%, p = 0.03). Women 45-54 yr. showed a 5 yr. overall and relative survival of 51 and 54 % respectively, and a 10 yr. survival of 40%.

Discussion: Observed relative survival in this cohort (64% in 5 years) reveals quite similar estimates compared to such observed in Slovenia (64%) and Poland (60%) , but substantially lower than ascertained in the same years in France (80%), Finland (78%) and Germany (72%). The observed differences may probably be related to the advanced staging at diagnosis in Goiania in the late eighties, a condition often associated to poor results following therapeutic intervention on breast cancer patients.

310 Abnormal expression of G1 cell cycle proteins in breast tumors of African American women.

Porter PL, Lund MJ, Eley JW, Coates RJ, Flagg EW, Liff JM, Lin M-G, Yuan X, Schmidt J, Stafford S. Fred Hutchinson Cancer Research Center, Seattle, WA; Emory University, Atlanta, GA; Center for Disease Control, Atlanta, GA

Background: African Americans experience decreased breast cancer survival compared with white Americans. Differences in stage account for a portion of the survival differences. However, differences in survival remain within stage and treatment groups. G_1 cell cycle abnormalities are common in tumors and are often associated with poor survival. We report stage-adjusted differences in expression of cell cycle regulatory proteins in breast tumors of African American and white women in Atlanta. **Methods:** Breast tumors from 124 African American and 397 white women enrolled in a case-control study of breast cancer risk (Women's Interview Study Of Health (WISH)), aged 21-54 and diagnosed between 1990 and 1992, were pathology reviewed and evaluated for expression of ER, PR, Ki-67, p53, cyclins E and D1, p27, p16, pRb, and p21 by IHC. Age at diagnosis, tumor size, clinical stage and lymph node status were obtained from the Atlanta SEER Registry and medical records. Logistic regression was used to assess the age and stage adjusted relationship between race and tumor characteristics. **Results:** African Americans were more likely to be diagnosed at young age and late stage, have large tumors, exhibit metastases, and have tumors of high grade and high mitotic index. Age, grade, and mitotic index remained distinguishing characteristics between racial groups after statistical adjustment for stage. Tumors of African Americans were also more likely than those of whites to be ER (OR=2.57, 95%CI 1.60-4.12) and PR negative (OR=2.76, 95%CI 1.74-4.37); to over-express cyclin E (OR=4.26, 95%CI 2.00-9.08), p16 (OR=2.57, 95%CI 1.55-4.27), and p53 (OR=1.69, 95%CI 1.04-2.75); and to lack cyclin D1 (OR=0.51, 95%CI 0.31-0.85). **Discussion:**The contribution of socio-economic and biologic differences to decreased survival of African Americans from breast cancer is under active debate. Our findings of increased cell cycle abnormalities in tumors of African American women indicate that specific tumor cell defects may be associated with race. Future studies will determine how socioeconomic and breast cancer risk factors may contribute to these biologic differences.

311 Race—not socioeconomic status—relates to breast cancer tumor size.

Crowe JP, Patrick RJ, Rybicki LA, Grundfest-Broniatowski SF, Kim JA, Lee KB. Cleveland Clinic Foundation, Cleveland, OH

Background: Many studies use soft data to support the premise that black patients present with more advanced breast cancer relative to white patients because black patients are more often in a lower socioeconomic status (SES). We analyzed our data to determine if tumor size and race correlate with SES for patients diagnosed with breast cancer.

Methods and Materials: Using data collected prospectively in our breast center registry and 2000 Census Track data, we compared tumor size and race with SES for 1660 patients who were either black (n=229) or white (n=1431). We defined a low SES (median household income < $41,200), a middle SES ($ 41,200-57,350) and an upper SES (> $57,350). We hypothesized that black patients in a lower SES would have larger tumors relative to white patients. The Chi-square test, t-test, and logistic regression were used for statistical analysis.

Results: Of 1660 patients 229 (14%) were black and 1431 (86%) were white. Black patients represented 30% (n=165) of 552 low SES patients, 10% (n=53) of 554 middle SES, and 2% (n=11) of 554 upper SES. Overall, black patients were more likely than white patients to have tumors > 2 cms (35% vs. 27%; P=0.015), and to have a lower SES (median household income $32,912 vs. $53,078; P<0.001). Using logistic regression analysis to adjust for SES, we found that black patients are 1.4 times more likely than white patients to present with tumors > 2 cm (P=0.024). In the low, middle, and high SES groups, model-based probabilities of presenting with large tumors are 35%, 36%, and 34% in black patients and 27%, 28%, and 26% in white patients.

Discussion: Previous studies suggest that black patients present with larger tumors relative to white patients because, overall, black patients have a lower SES. While our data confirm the majority of black patients (72%) have a lower SES, the racial disparity in SES does not account for the difference in tumor size at presentation between black and white patients. Tumor size is consistently larger for black patients irrespective of SES.

312 Racial disparity in the quality of breast cancer adjuvant chemotherapy.

Griggs JJ, Sorbero MES, Stark A. University of Rochester, Rochester, NY; Henry Ford Health System, Detroit, MI

Breast cancer mortality rates are consistently higher in African-American women than in Caucasian women. This disparity is outcome is not fully accounted for by differences in stage or biology of the disease. **Objective**: The purpose of this multicenter study was to determine whether or not there are systematic differences in the quality of adjuvant chemotherapy (defined as dose proportion and dose intensity) received by African-American and Caucasian women. **Methods**: We performed detailed audit of medical oncology chemotherapy treatment records in 435 subjects (91 African-American and 344 non-Hispanic Caucasian) who received adjuvant chemotherapy with a cyclophosphamide-containing regimen between 1985 and 1995 in 10 treatment sites in two cities (Rochester, New York and Detroit, Michigan). Data collected included demographic and clinical characteristics and information regarding height, weight, chemotherapy doses, dates, dose reductions, white blood cell counts, side effects, and complications. Chemotherapy dose proportion (actual/predicted doses) and dose proportion (which incorporates the time required for completion of chemotherapy) were determined for each drug and for the regimen. Multivariate regression models were designed to determine the impact of self-defined ethnicity, age, socioeconomic variables, coexisting medical conditions, regimen, site, and year of treatment on dose proportion and dose intensity. **Results**: African-American subjects received significantly lower dose proportion and dose intensity than Caucasian women. The mean dose proportion among African-American women was .79 compared with .85 among Caucasian women (p = .02). The mean dose intensity among African-American women was .73 compared with .81 among Caucasian women (p = .004). In multivariate analyses, race/ethnicity was an independent predictor of lower dose proportion (p = .03) and dose intensity (p = .005). **Conclusion**: The disparity in breast cancer outcome between African-American women and Caucasian women may be due in part to the receipt of suboptimal adjuvant chemotherapy among African-American women.

313 Improving follow up care to minority women with breast abnormalities.

Battaglia TA, Chapman CE, Han J, Freund KM. Boston Medical Center, Boston, MA

Background: Despite increases in breast cancer screening with mammography, racial disparities in breast cancer outcome persist. Our previous work has shown significant delays in follow up care after abnormal breast cancer screening in our population of predominantly low income, minority women. We sought to evaluate an outreach intervention to improve the timeliness of follow up care through the use of a case coordinator model.
Materials and Methods: We conducted a focused needs assessment, interviewing women who arrived and did not arrive to their clinical visit at our Breast Health Center to evaluate an abnormality detected by screening mammography or clinical breast examination. We identified communication regarding the appointment as a major barrier to keeping the appointment. A full time case coordinator, hired through a grant from the Avon Foundation, was charged with contacting all patients herself or through interpreter services, reviewing the plans for her appointment, and assisting her with logistics such as transportation, finding prior mammograms, and insurance issues.
Results: Prior to implementation of the intervention, we evaluated 390 consecutive women referred from January- June 2000. 50% were between ages 40 - 64, 70% were minority (51% black), 73% had Medicaid or no insurance, 19% were non proficient in English and 51% were referred from neighborhood health centers. 36% of all women did not arrive for the office visit. Younger women (OR 1.9, CI 1.1- 2.9), and Medicaid or no insurance (2.0, 1-1-3.4) were associated with inadequate follow up. Following implementation of the intervention, we reevaluated 651consecutive women referred from March 2001 - January 2002. Only 17% failed to arrive for their appointment ($p < .001$).
Discussion: Significant delays in evaluating abnormal breast screening studies may contribute to racial disparities in breast cancer outcomes. More intensive case coordination which is not routinely covered by health insurance is necessary to ensure adequate follow up of at risk populations.

314 Fundamental prognostic indicators for breast cancer in black and white patients—there is a difference.

Crowe JP, Patrick RJ, Rybicki LA, Grundfest-Broniatowski SF, Kim JA, Lee KB. Cleveland Clinic Foundation, Cleveland, OH

Background: The purpose of this study was to determine if race correlates with initial tumor characteristics and prognostic factors for black and white patients who seek treatment at a large multidisciplinary breast center.
Methods and Materials: We hypothesized that black patients would present with tumors having less favorable prognostic markers relative to white patients. Using data collected prospectively in our breast center registry we compared tumor size, node status, TNM stage and hormone receptor status for 1660 patients who were either black (n=229) or white (n=1431). The Chi-square test was used for statistical analysis.
Results: Of 1660 patients 229 (14%) were black and 1431 (86%) were white. Black patients were more likely than white patients to present with tumors > 2 cms (35% vs. 27%; P=0.015), to have positive axillary nodes (37% vs. 29%; P=0.033), to have TNM Stage II, III, or IV disease (48% vs. 38%; P=0.005), and to have hormone receptor negative tumors (35% vs. 17%; P<0.001).
Discussion: Previous studies have questioned if breast cancer presents differently in black and white patients, and numerous variables are considered to either support or deny a position. We demonstrated that black patients are more likely to be diagnosed with larger tumors, positive axillary nodes, later stage breast cancer, and hormone receptor negative disease relative to white patients. These fundamental differences are indicative of poorer prognosis.

315 Factors associated with heightened perception of risk among African-American women with a family history of breast cancer.

Simon MS, Du W, Greb A, Berry L. Karmanos Cancer Institute/ Wayne State University, Detroit, MI; Center for Molecular Medicine and Genetics, Detroit, MI

Background: Family history (FH) of breast cancer remains the most important predictor of lifetime breast cancer risk, and a major determinant affecting referral for cancer risk assessment and genetic counseling. Uptake of breast cancer prevention options is less common among African American (AA) compared to white women and is partially associated with a lower perception of cancer risk.
Material and Methods: To better understand predictors of risk perception among AA women with a family history of breast cancer, we conducted a survey of 100 women age 18 years and older who had at least one first-degree relative with breast cancer. Participants were recruited from among family members of breast cancer patients seen at a large urban university based medical center, and from the surrounding metropolitan area.
Results: Predictors of heightened breast cancer risk perception included college education (65% of college graduates vs.38% of non-college graduates had a heightened perception of risk, p=0.007), concerns about breast cancer (71 % of women who were very concerned vs. 35% of women with moderate, slight or no concern, had a heightened perception of risk p=0.004) and intrusive thoughts about getting breast cancer (69% of women who had frequent intrusive thoughts vs. 43% of women who had infrequent intrusive thoughts had a heightened perception of risk, p=0.018). Factors associated with family cancer history including type of first degree relative (mother or sister), number of affected first-degree relatives, death of relatives with breast cancer, and level of involvement with affected relatives did not seem to be correlated with a heightened risk perception.
Discussion: In order to promote increased utilization of cancer risk assessment and genetic counseling among AA women at high risk of breast cancer it is important to educate women about the association between family cancer history and cancer risk.

316 Widening the scope of oncology malpractice suits to include HMOs and health plans: how lessons learned from previously allowed managed care litigation may impact the outcomes of future breast cancer complaints.

Goebel RH. Long Beach Community Cancer Center, Long Beach, CA

In many jurisdictions, it has recently become much easier for patients to successfully sue their HMOs for malpractice. It is therefore important for both physicians and health plan administrators to become aware of their potentially increased liability exposure as a result of this. To better understand this a litigation analysis was performed using oncology cases in LEXIS and WESTLAW. A total of 41 cases were found in which HMOs or health plans were allowed to be sued for alleged errors despite existing statues favorable to health plans. There were 31 plaintiff awards totaling $13,039,189. Breast cancer patients constituted the largest group of plaintiffs (N = 20). Other plaintiff diagnoses included Brain Tumors (N = 4) Rectal cancer (N = 3), Colon Cancer (N = 3) and Prostate Cancer (N = 3). Although the majority of suits alleged failure to diagnose cancer or to timely refer for treatment (N = 30), there were nevertheless 9 suits alleging that proper treatment had been withheld. These included 2 cases involving bone marrow transplants and one where standard chemotherapy was not advised. The outcomes of this sample of cases suggest that the negligent acts of physicians diagnosing and treating cancer patients in a managed care setting can be effectively transferred to the affiliated managed care entities. Such entities should be cognizant of this in their risk management activities.
The current position of statutory and case law in several jurisdictions (including Texas, California and the Federal Government) will be summarized. The current laws in these jurisdictions will be applied to fact patterns from several representative Breast Cancer Lawsuits to compare and contrast the possible outcomes of breast cancer complaints using alternative rules of law.

317 Physician and patient barriers to breast cancer clinical trials.

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Background: Clinical trials (CT) are essential to develop and test novel therapies, yet only 2-3% of women with breast cancer enroll in trials. Prior studies describing patient and physician barriers to CT participation have not addressed concerns specifically when patients are eligible to enroll. Also, little is known about attitudes regarding complementary and alternative medicine (CAM) clinical trials.

Methods: Separate questionnaires were offered to patients with newly diagnosed or recurrent breast cancer treated at UCSF and to physicians specializing in the care of breast cancer patients in the San Francisco Bay Area in 1997 and 2000. Responses were recorded on a 5-point Likert scale measuring agreement with statements about CT. Interventions aimed at patients and physicians to increase their knowledge and support for breast cancer CT were initiated in 1997. Responses to the 1997 and 2000 surveys were compared, and correlation between perceived barriers and patient and physician demographics were explored.

Results: The strongest barrier to CT participation in patients was reluctance to be randomized. Others included concern about extra time for tests and visits and the possibility of worse side effects with the experimental arm. Patients agreed that CAM should be studied, and that CT can combine CAM and conventional treatments. However, older patients felt that CAM was still too unknown to be tested in a CT. Lower stage patients were more concerned with loss of control. Physician barriers included randomization and the extra staff time and costs of CT enrollment. Medical oncologists felt more strongly than other physicians that eligibility criteria were too strict. Private practice physicians had more concern that CT might delay therapy.

Discussion: In general, both physicians and patients developed more favorable views of CT between 1997 and 2000 based on the average response score to the entire questionnaire. Patients and physicians approved of studying CAM in CT, but there were differences based on age and type of practice. These results indicate that attitudes towards CT are significantly affected by patients age and stage of disease. Efforts to improve enrollment should focus on these individual differences as well as uneasiness with the randomization process. Interventions that address population-specific barriers might effectively increase CT participation.

318 Why do patients accept or refuse to enter into randomized clinical trials for metastatic breast cancer therapy?

Protiere C, Viens P, Camerlo J, Braud AC, Cappiello MA, Gravis G, Viret F, Moatti JP, Genre D. Institut Paoli Calmettes, Marseille, France

Background: Very few information were available on patients' (pts.) motivations to accept to enrolled themselves into randomized clinical trials (RCT) and on pts who refused. The questions we want to answer with this study are 1) what are pts' motivations? 2) are pts who refused to enter into RCT different from the ones who accept? and 3) would these pts prefer to be more involved in the medical decision processes (MDP)?

Material and Methods: All pts who met eligible criteria to enter into 2 RCTs for metastatic breast cancer at the Institut Paoli-Calmettes were included in the study. One compared 2 chemotherapy regimen for first line disease, the 2nd compared Thalidomide vs. placebo for maintenance of response. An auto-questionnaire was elaborated to elicit pts' motivations for participating, or not, in RCT, their characteristics and their ideal degree of participation in the MDP. The questionnaire was reviewed by a committee of pts and filled out after deciding to enter or not in the RCT.

Results: 34 pts were included in the study; there was a higher rate of acceptance in the trial providing active treatment in the two arms (90%) compared with the one with a placebo arm (60%). The most important reasons for accepting to enter into RCT were 'to think having a best medical follow up by participating in a trial' (30%) and 'trust in the doctor' (25%). The most important reason for refusing RCT entry was 'the experimental treatment is associated with too much risk' (56%). Most of pts (59%) have considered the providing information clear and sufficient. A significantly higher proportion of participating pts declared they would have prefer to choose themselves their treatment (82%) compared with those who have refused (33%, $p<0.037$); however, all the pts who have refused considered suitable a shared MDP. 76% of participating pts have expressed a preference for the experimental treatment.

Discussion: The rate of 29% of refusal is mainly due to the RCT with a placebo arm showing the difficulty to include placebo for vital disease. One could be surprised that pts who refused RCT were not the ones who are expected for a higher pts empowerment.

319 Overcoming barriers to care: the AvonCares program.

Grober SE, DeBor M, Levy J, Blake M, Blum D. Cancer Care, Inc., New York, NY; Avon Foundation, New York, NY

Background: The AvonCares program was established in 3/2000 with a $2 million grant from the Avon Foundation to Cancer Care, Inc, a 57 year old non-profit organization dedicated to providing free professional support services to people with cancer and their families. The program offers financial assistance for diagnostic tests, transportation, childcare, and escort services to women with breast, ovarian, and cervical cancers, and for those needing pre-diagnostic testing. Social workers assess the clients' needs, and offer financial assistance of up to $1000 along with counseling, education, and help with practical problems.Cancer Care, Inc. has reached out to more than 20,000 organizations throughout the United States to inform them about AvonCares, and provided services to 7000 women in 50 states since program inception. Not only have these women received direct financial assistance, but also through the connections forged with Cancer Care as a result of this program, they have benefited from access to free professional counseling, educational programs, and referrals to resources that would not have been readily available to this underserved, underinsured population.The clients served by AvonCares were ethnically diverse with 62% white, 18% Latino, and 18% African American. Ages ranged from 22-81, with 66% between 32 and 60.

Methods: Service utilization patterns of women who received funding from this program were analyzed to identify additional unmet needs fulfilled by this partnership.

Results: Education and information (49%); counseling (28%); and referral to additional resources (19%) were the most frequent needs of these underserved women. Ninety percent (90%) of sampled program participants had serious ongoing concerns about paying for care, which is not surprising because we found that, overall, 82% were not working and 37% had no insurance.

Discussion: Financial, emotional, and practical barriers can prevent women from receiving needed health care. The Avon Cares program of Cancer Care provides financial assistance and support services to overcome these barriers, and illustrates how financial assistance programs can help to engage underserved populations in more actively addressing the multidimensional needs of cancer patients.

320 Breast cancer on the internet. What people really want to know.

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Background: We recognized that (1) users less sophisticated in web technology and medical terminology often begin their cancer information searches on popular search engines rather than on prominent cancer information sites, and (2) this was how many visitors came to our site. Because we want to understand better the actual queries of those users, we evaluated the records of a natural language processing search engine, which permits users greater latitude in crafting questions than key word searches and gives a clearer picture of what lay users seek. Therefore, NCI and AskJeeves, Inc. developed a methodology to capture, sample, and analyze 3 months of cancer-related queries on the Ask.com web site, a prominent US natural language processing search engine, which receives over 35 million queries per week. **Materials and Methods:** Using a benchmark set of 500 terms and word roots supplied by NCI, Ask.com identified a test sample of cancer queries for 1 week in August 2001. From these 500 terms, only 37 appeared more than 5 times per day over the trial week. Using only these 37 terms, 204,165 instances of cancer queries were found in the Ask.com query logs for June, July and August 2001. Of these, 7500 individual user questions were randomly selected for detailed analysis. Considering multiples of the same question, these 7500 represented 76,077 actual queries or 37% of the total 3 month pool. These 7500 were analyzed by search term, organ site, subcategory and style. **Results:** The 3 most frequent terms/roots were *cancer* (56.93%), *tumor* (8.14%), and *carcino* (3.82%). The most frequent organ site was breast (15.07%), followed by skin (12.36%), lung (8.45%), colorectal (7.68%) and lymphoma (6.25%). Among breast queries (100%), 10 subcategories were identified: general information (49.23%), symptoms (12.79%), treatment (8.20%), media/organization (6.16%), causes/risk/links (5.65%), diagnosis and testing (5.41%), statistics (3.94%), pictures (3.24%), type/form of cancer (3.12%), and definition (2.27%). Within the general information category (100%), the top 5 were unspecified (83.79%) e.g., "Where can I find information on breast cancer?", male (5.52%), stages (4.79%), coping /support groups (2.54%), and research/journals (2.45%). More granular data from subcategories will be presented. There were few queries about breast clinical trials, alternative therapies, or mammography. **Discussion:** The most frequent topics of general public queries reflect and fluctuate with reporting of important new research studies or news controversies. Our data reflect only what was queried in the study dates. We have no information about what users picked when search results were returned at Ask.com. Based on these data, we hope to pilot natural language searching on select NCI web sites in order offer users easier, more customized search results. We also plan to consider these data when presenting information to users of our sites.

321 Usability of breast cancer web sites.

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Purpose: To evaluate the usability of health-related information for patients with breast carcinoma available on the Internet, measured as average search engine rank (ASER), average hit count (AHC), average number of links (ANL) and relevant information density (RID).

Methods: Using a special software tool all the hits from 19 search engines were collected, providing a result of 10.616 hits for the keywords "breast cancer", "Brustkrebs" or "Mammakarzinom". These hits were checked for dublettes and sorted in a database (date: 2001-03-01, 19:36). Then all URL addresses were visited and analysed with a specially developed questionnaire.

Results: RID: Of 3.812 addresses in German language only 65 (1.7%) and of 6.804 English language search results 131 (1.9%) contain detailed information on Breast Cancer treatment. Information on special parts of the disease (e.g. mammography screening) is given in 8.6% (German sites) and in 13.7% (English & American sites). Owner of the relevant websites are institutions or organizations in 45%, industry in 41% and private in 14%. Additional web usability parameters: AHC 1,58, ANL 38,8, dead links 5,3 %, ASER 71,7, mean size of sites is 77,8 kB (download time with analog modem more than 21,6 seconds).

Conclusions: Just a few breast cancer web sites contain exact information about treatment options. These sites are hard to find in this mass of available search results. Web usability of informations for breast cancer patients is not sufficient (dead links, uncomfortable download time).

322 Clinical care assistance: serving underserved women.

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Background: UCI is the only academic medical center serving the 2.8 million residents of Orange County, CA. Despite an annual median household income of $49,583, 11% of Orange County residents are below poverty and 17% of adult women in Orange County have no health care coverage. The Avon Foundation awarded UCI $4.2 million over three years to advance breast cancer research, and to assist underserved women in need of breast health care and breast cancer resources.

Program Description: The Clinical Care Assistance Program provides assistance to uninsured and underinsured women in Orange County. Through collaboration with state and federal programs, local organizations and other medical facilities, we offer access to breast cancer education, screening, diagnostic work-ups, treatment, support and non-medical resources, such as payment of rent, groceries, utilities, and wigs. The range of services also provides a framework encouraging these women to participate in clinical trials that otherwise might not have been possible.

Results: 70 women have been assisted since the program began in June 200. Many of them have incomes too high for state/federal assistance, but not high enough to purchase health insurance. Approximately 5% of the requests for assistance have been for screening, 30% for diagnostic work-ups, 45% for treatment, and 24% for other non-medical services (duplicated numbers as some women request multiple types of assistance). About 55% of the women are over 40. Affiliated programs: Breast and Cervical Cancer Control Program, Susan G. Komen Foundation, Breast and Cervical Cancer Treatment Program, Breast Cancer Angels, Breast Cancer Survivors, AvonCares, and pharmaceutical programs. We also have 18 women participating in breast cancer treatment studies, 40 in our MRI breast study, and approximately 150 in our laser breast imaging study.

Discussion: UCI would like to share this program with conference participants. Orange County has strong breast cancer education/advocacy support, and because of consistent networking, our patients benefit from extensive community resources. We will provide the history and background of our program, a description explaining how the program works, and statistical results to show the impact on our facility and our community.

323 Effective physician involvement of advocates in cancer patient education.

Hetrick VR, Feldman NR. You Are Not Alone, Los Angeles, CA; University of California Los Angeles, Los Angeles, CA

With today's current third-party payer environment, physicians are constantly faced with time pressures to see more patients during their clinic hours. As a consequence, in spite of their best efforts, physicians must diagnose, treat, educate patients and families, and answer questions in a very limited period of time. An effective way to optimize physician time with patients is to find a way to help them educate patients. Well-trained patient advocates can uniquely cooperate with physicians to provide that assistance. Since 1993, the authors have been cooperating to educate patients in two areas: inflammatory breast cancer and/or high-dose chemotherapy(HDC). Here, we show the model we have worked out to provide education such that the information presented to patients is both accurate and timely. We have developed a program to train advocates as peer counselors. Advocates are individuals who have been treated for inflammatory breast cancer or who have received high-dose chemotherapy and who have completed treatment at least one year before they volunteer. Those who wish to participate undergo a formal training program and a one-year mentorship. Then, they are referred as peer counselors to patients who are being treated at various institutions in southern California. Advocate education consists of formal classroom instruction in the basics of genetics, molecular biology, and clinical and psychosocial aspects of oncology, Q and A with clinical oncologists and a psychotherapist who specializes in treating cancer patients, lectures on services available through various medical centers and patient contact information for those services. More than 25 patient-written essays, reviewed by You Are Not Alone's Medical Advisory Committee, are available on the You Are Not Alone website at http://www.yana.org with practical information about cancer treatment, survival, and end-of life issues. In our particular situation, all of the advocates are volunteers, though we know of several institutions where the advocates are compensated. Patients benefit because they develop a better understanding of their disease in a less time-pressured setting. Physicians benefit because their time with patients can be used to maximum effect at a relatively low cost to their institutions.

324 Breast cancer advocates create an evidence-based patient-centered guide to quality breast cancer care by working with diverse scientific, medical and educational experts.

Brunswick C, Collina SH, Hain C, Visco FM. National Breast Cancer Coalition, Washington, DC

Background: The National Breast Cancer Coalition (NBCC) is a grassroots organization representing millions of people affected by breast cancer. Driven by the passion of educated activists, NBCC provides an evidence-based patient perspective that is so often missing from policy debates. One goal of NBCC is to describe a vision of quality breast cancer care and ensure that everyone who needs it gets it. To achieve this, NBCC is working to create an environment where evidence-based health care is understood and expected. Currently there is not a clear understanding of the value of a science-based approach to health care, and its benefits for patients. And there is no national consensus on what makes breast cancer care "quality" care. Still, recent studies have begun to reveal significant problems with the quality of care delivered in the U.S.

Materials and Methods: To create a vision of quality breast cancer care, NBCCF brought together a group of visionaries from within and outside of the world of breast cancer. Participants included breast cancer activists, business leaders, government officials, historians, industry representatives, members of the media, policy professionals, scientists from diverse fields and health care providers. Through "blue sky" exercises, participants were asked to forget about the limitations of the "real world." From this work NBCC developed six core values–six components of quality breast cancer care: access, information, choice, respect, accountability, and improvement. Building on this vision, NBCC created a guide to breast cancer care, which provides a roadmap for understanding and getting patient-centered evidence-based quality care. NBCC then solicited feedback from hundreds of breast cancer activists, health care providers, payers and users, researches, and edited the language to "plain English" (8th grade level).

Results: This collaborative approach to creating the Guide — a process which brought in so many different perspectives — created a thoroughly patient-centered, evidence-based vision of quality care that can be a model for other diseases.

Discussion: Other educational materials on breast cancer mostly focus on stages and treatment choices, how to talk to your doctor, or how to find emotional support. NBCC's Guide to Quality Breast Cancer Care provides a unique focus on what quality care looks like, and specific advise on what can be done right now to improve breast cancer care. It is hoped that this tool will help individuals facing breast cancer, encourage a health care environment where scientific anaylsis is at core of health care decisions, and serve as an advocacy tool for improving the quality of breast cancer care available across the country.

325 NBCCF's beyond the headlines education program.

Platner JH, Koppelman LF. National Breast Cancer Coalition Fund (NBCCF), Washington, DC

NBCCF is committed to fostering public policies that increase meaningful breast cancer research, improve access to quality breast cancer care, and expand the role of breast cancer activists in making breast cancer policy. To further these goals, NBCCF has launched a program to bring an unbiased advocate perspective to emerging breast cancer issues.

Lay and scientific press reports typically discuss emerging breast cancer issues from the perspective of industry, science or policy makers. NBCCF's program analyzes these issues and brings an informed, educated consumer advocacy perspective to the debate, in the form of position statements and fact sheets. These educational materials are developed by NBCCF staff and board members who review all pertinent research evidence and solicit input from both scientists and advocates. The materials are then discussed, edited, and approved by NBCCF's Board of Directors. The final products are posted on NBCCF's website and distributed by NBCCF's national network of breast cancer advocates. They are reviewed on a monthly basis, and updated as new information and evidence becomes available. NBCCF's member organizations often distribute these educational materials to their individual members, and/or use the information to create their own educational materials.

NBCCF's position statements and fact sheets are based on the best available research evidence as well as a broad public health perspective. These materials present all sides of an issue and explain the political context and scientific background in lay-terms. Two examples of NBCCF's educational materials include 1) a position statement on the prophylactic use of tamoxifen and raloxifene, and 2) a fact sheet on the recent controversy surrounding mammography. Both of these papers present an honest and complete assessment of the risks and benefits of each public health intervention.

Most breast cancer issues are complex. NBCCF's educational materials discuss the pros and cons of each issue so that readers can better analyze the scientific and medical information that they obtain from the media and other sources. NBCCF believes that women and policy makers must be given all of the information so that they can make truly informed decisions.

326 Addressing breast cancer risk factors in rural African American churches.

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Purpose: The purpose of this project was to form a partnership with an African American church-based coalition (the Alliance of Black Churches) to develop and pilot test a culturally competent breast health promotion exercise videotape targeted to rural African American women from Central Virginia. Description of study: A questionnaire was developed and administered to members and associates of the Alliance in order to assess the needs of the target population related to breast cancer risk reduction behaviors, including exercise and a healthy diet. Questionnaire results, in association with input from Alliance members, informed development of the videotape. The completed videotape was pilot tested among 12 members and associates of the Alliance over a 12-month period. Results: A total of 50 women responded to the initial questionnaire assessing the needs of the target population. Of these, 55% reported that they exercised during their leisure time. The majority of respondents reported that, on average, in a week they consumed six or less fruits (56%) and six or less vegetables (72%). Nearly half reported that they ate red meat 1-2 times weekly. Most respondents recognized the importance of exercise for one's physical and emotional health. Input concerning the structure and content of the exercise videotape was sought during a focus group with members of the Alliance. Suggestions included: using music with a beat and gospel music; involving women of different ages and races; involving women of all sizes; and including men. Results of a focus group held following the distribution of the videotape revealed that the tape was seen as a useful resource for the 12 women involved with its pilot test. Implications: The success of this work was the direct result of a partnership with an existing church-based organization. This partnership resulted in the development of a valued health promotion tool designed to positively influence breast cancer-related health risk behaviors among an African American population in rural Central Virginia.

327 Phase III study of docetaxel 100 versus 75 versus 60 mg/m^2 as second line chemotherapy in advanced breast cancer.

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Objectives: The primary objective was to determine if a dose-response relationship exists within the docetaxel dose range 60-100 mg/m^2 and to compare response rate (RR) between the 3 arms. Comparison of time to progression (TTP), overall survival (OS) and safety were secondary.

Results: 527 patients (pts) were included; median (med) follow-up was 30 months (mos). Pts were treated until progressive disease or unacceptable toxicity; no maximum number (nb) of cycles (cy) was pre-specified. Main pt and tumor characteristics were balanced between groups. Med (range) nb of cy in the 3 groups (100/75/60 mg/m^2) was 6 (1-24) / 6 (1-39) / 5 (1-43), med relative dose intensity was .97/.98/.99. In evaluable pts there was a significant dose-response relationship (p=0.007), and RR was different between groups (p=0.017). TTP also showed a significant dose-response relationship (logrank p=0.014, med (weeks): 18.6/13.9/13.7).

	100 mg/m^2	75 mg/m^2	60 mg/m^2	
Randomized/treated	188/186	188/188	151/150	
Evaluable	139	146	122	
Complete RR	6.5%	1.4%	2.5%	
Overall RR	36.0%	23.3%	22.1%	p=0.017

Similar results were seen in the ITT population. No significant difference between groups in OS (med (mos): 12.3/10.3/10.6). Discontinuation due to toxicity: 17/7/5%. Main grade 3/4 related events by pt: neutropenia 93/84/76%, febrile neutropenia 14/7/5%, infection 7/3/2%, stomatitis 5/2/1%, diarrhea 5/4/1%, fluid retention 6/4/1%, neurosensory 4/2/1%. Toxic death: 1pt (grade 4 diarrhea at 60 mg/m^2).

Conclusion: There was a significant dose-response relationship in the range 60-100 mg/m^2, and RR differed between the groups. Overall the 100 mg/m^2 group had the best efficacy and was associated with higher but manageable toxicity.

328 Epirubicin containing regimens as Ist line chemotherapy in advanced breast cancer are safe and active in patients treated with adjuvant epirubicin.

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Adjuvant epirubicin seemed to be associated with decreased efficacy of Ist line Epirubicin in MBC. We analysed the effect of adjuvant epirubicin on the activity of Epirubicin/Paclitaxel CT in MBC. Data from 291 patients enrolled in 5 trials between 1994 and 2001 were analysed. Ist line CT consisted of: Epirubicin 90mg/sqm + Paclitaxel 200mg/sqm (150 pts), Gemcitabine 1000mg/sqm + Epirubicin 90 mg/sqm + Paclitaxel 175mg/sqm (64 pts), and Epirubicin 120mg/sqm x 4 courses followed by Paclitaxel 250mg/sqm x 4 courses (77 pts). One-hundred-one patients (35%) were chemonaive, 109 (37%) received CMF and 81 (28%) the FEC regimen. Patients characteristics were: median age 55 years (range 30-73), PS 0 (0-2); dominant metastatic sites were viscera in 206 patients, bone in 46 and soft tissue in 39. ER status was positive in 150 patients, negative in 69 and unknown in 72. ORR was 69% for prior adjuvant Epirubicin, 63% for prior CMF and 70% for no prior CT (p=0.5). CR rate was 14% both in case of prior CMF or anthra-based CT and 22% for no prior adjuvant CT; however, this difference was not statistically significant (p= 0.1). On a multivariate analysis the probability of achieving a response was not affected by prior adjuvant Epirubicin. Median PFS was 10.2 months for prior adjuvant Epirubicin, 11.0 months for prior CMF and 12.5 months for no prior adjuvant CT (p=0.3). Median OS was 20.2 months for prior adjuvant Epirubicin, 23.8 months for prior CMF and 27.4 months for no prior adjuvant CT (p=0.6). By multivariate analysis prior adjuvant epirubicin was not associated with OS and PFS.

In conclusion our data indicate that the activity and efficacy of first-line chemotherapy for metastatic disease including epirubicin and paclitaxel is not significantly affected by the administration of prior adjuvant epirubicin. For this reason, re-treatment with epirubicin should be considered for all patients with MBC; moreover, in the same patient we previously demonstrated that Epirubicin can be safely administered up to cumulative doses of 900 mg/sqm, thus allowing both adjuvant and metastatic administration, without increase in cardiotoxicity (Gennari et al, JCO 99).

329 Smoking status correlates with myelosuppression in patients treated with gemcitabine-docetaxel.

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Seventy-nine patients with previously treated metastatic NSCLC and breast cancer were treated with gemcitabine (800 mg/m^2 d 1, 8, and 15) and docetaxel (100 mg/m^2 d 1) every 28 days, on 2 separate phase II trials conducted by the Columbus CCOP (Annals of Oncology 12:89 and 12:1259, 2001). The incidence of grade 4 neutropenia (ANC < 500) on day 8 of cycle 1 was higher in the breast cancer patients (72% vs 40%), but the cause of the difference was unclear.

Another cohort of 11 breast cancer patients was treated "off-study" with this regimen, but with a lower dose of docetaxel (75 mg/m^2). Seven experienced grade 4 ANC on day 8 of cycle 1. An association with smoking status was noted: severe neutropenia occurred in 6 of 7 non-smokers, but in only 1 of 4 smokers (Laufman, UICC abstract # P 177, 2002).

Therefore, a retrospective analysis of the 2 phase II trials was done, to correlate smoking status with grade 4 ANC on cycle 1 day 8. Information on current smoking status was available on the original charts of 70 patients. Grade 4 ANC occurred in 37 of 46 non-smokers (80%), compared to 9 of 24 smokers (38%), p< 0.0005, Fisher's exact test. For breast cancer patients, grade 4 ANC occurred in 23 of 26 non-smokers, compared to 5 of 8 smokers. For NSCLC patients, grade 4 ANC occurred in 14 of 20 non-smokers, compared to 4 of 16 smokers. A multivariate analysis of these data will be presented. A plausible mechanism for this observed difference is that nicotine may up-regulate cytochrome P 450 3A4, an enzyme involved in docetaxel metabolism. If these preliminary retrospective findings are confirmed, the implications are great. Non-smokers may need lower doses of chemotherapy to avoid severe myelosuppression, and smokers may potentially need higher doses to achieve similar anti-neoplastic effects.

330 Randomized phase II study of docetaxel vs. epirubicin/ cyclophosphamide to optimize first-line therapy of metastatic breast cancer (MBC): preliminary results of the TIPP study.

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Background: Docetaxel is the most active single agent in the treatment of MBC and may produce higher response rates than current standard regimens. To define the therapeutic role of docetaxel more clearly, we initiated a single-center randomized phase II study of docetaxel versus epirubicin and cyclophosphamide (EC). **Methods:** Patients (pts) with MBC, no prior chemotherapy for metastatic disease, and WHO performance status 0-1 are eligible for the study. Accrual of 40-60 pts is planned. Docetaxel is given at 100 mg/m^2 along with standard oral dexamethasone premedication. Patients in the EC arm receive epirubicin 90 mg/m^2 as an intravenous bolus followed by an intravenous infusion of cyclophosphamide 600 mg/m^2 . In both arms, cycles are repeated every 3 weeks to a maximum of 6 cycles. **Results:** To date, 26 pts have been randomized (docetaxel 11; EC 15), and 12 pts (docetaxel 6; EC 6) have completed chemotherapy. Two EC pts died of progressive disease and another 2 EC pts showed progression under therapy. EC was given at full doses, whereas the dose of docetaxel was reduced to 75 mg/m^2 in one elderly frail pt. No dose delays were required. G-CSF for neutropenia prophylaxis was used in 9 patients (docetaxel 5; EC 4). A total of 32 cycles of docetaxel (mean=4.57) and 55 cycles of EC (mean=4.23) in 20 patients are currently evaluable for toxicity. Toxicity was mild to moderate except for alopecia. To facilitate comparison of toxicity between treatments, we calculated mean WHO grades per cycle. No significant differences for any specific toxicity were seen except for grade I cardiac toxicity in 6 pts (docetaxel 1; EC 5; P=0.036) and grade 1-3 neurotoxicity (docetaxel 6; P=0.028). Mean grade for all toxicities was <1 except nausea/vomiting in the EC arm (1.2) and alopecia in both arms (docetaxel 2.6; EC 2.7). **Conclusion:** Preliminary results indicate that both docetaxel and EC regimens are well-tolerated. More mature data, including response rates, will be available by October 2002.

331 Front line treatment of advanced breast cancer with docetaxel and epirubicin : Results from India.

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Introduction:

Docetaxel and anthracyclines have produced a high degree of activity in previously untreated patients with metastatic breast cancer(MBC). The efficacy of docetaxel as a single agent in second line therapy has been confirmed in large phase II studies. We did this retrospective study to find out the efficacy and safety of the combination of docetaxel and epirubicin as the first line chemotherapy in Indian patients with MBC.

Patients and Methods:

Data of 45 patients were analysed. Epirubicin dose was 60mg/m2 IV on D1 and docetaxel 75m/M2 IV as I hr infusion on D2. All patients included in this analysis had histologically proven advanced breast cancer with measurable disease and an ECOG performance status of 0-2. 41 patients were evaluable for response and toxicity.

Results:

Median age was 53 years (42yr-62yr). 27 (60%) had visceral disease; bone was the commonest site of metastasis. 41 patients were treated with 229 cycles of chemotherapy, the median number of cycles being 6. Of the 41 patients evaluable for response 7 (17.0%) achieved a complete response (CR). 23 patients (56%) had partial response. Over all response was 73%. Five patients had a stable disease (SD) and 6 had progressive disease. Grade III/IV neutropenia occurred in 67% and 35% had febrile neutropenia. Other hematologic toxicities were usually mild. Median over all survival was 11.5 months (range 4-26 months). Median time to progression was 9 months. 14 patients had progression of disease after achieving CR/PR/SD. Sites of failures were four each in brain and liver, two each in lung, bone and chest wall. 14 patients died.

Conclusion:

The combination of docetaxel with epirubicin is an active and safe regimen in the first line treatment of metastatic breast cancer. Only limiting factor for side use in developing countries would be the cost involved.

332 Multicenter randomized phase III trial of epirubicin plus paclitaxel vs epirubicin followed by paclitaxel: focus on cardiac safety.

Baldini E, Salvadori B, Prochilo T, Bolognesi A, Aldrighetti D, Venturini M, Rosso R, Carnino F, Visentini L, Gallo L, Mammoliti S, Giannessi PG, Di Marsico R, Bruzzi P, Conte PF. St. Chiara University Hospital, Pisa, PI, Italy; St.Raffaele Institute, Milano, MI, Italy; IST, Genova, GE, Italy; St.Anna Hospital, Torino, TO, Italy; Galliera Hospital, Genova, GE, Italy; Livorno Hospital, Livorno, LI, Italy; CBA, Genova, GE, Italy

Purpose: To evaluate cardiac safety of two different schedules of epirubicin (E) and pacitaxel (P) in metastatic breast cancer (MBC) patients (pts) randomized in a multicenter phase III trial.

Patients and methods: 202 MBC pts from 7 Institutions (6 Italian and 1 Spanish) were randomized to: A (E+P): E 90 mg/sqm + P 200 mg/sqm (3-hour infusion) d 1 q 3 wks x 8; B (E-P): E 120 mg/sqm d 1 q 3 wks x 4 followed by Paclitaxel (P) 250 mg/sqm (3-hour infusion) d1 q 3 wks x 4. Patients characteristics: median age 58 yrs (30-73), median ECOG PS=0 (0-2); prior adjuvant chemotherapy (CT) and/or hormonal therapy 60.4%, (anthracycline-based 25.2%). A total of 1296 cys were administered, median per pt 8 (0-8). LVEF was evaluated in 136 pts by bidimesional echocardiography performed on study entry, after 4 and 8 cys of CT both arms.

Results: Baseline median LVEFs were 60% and 65% arm A and B respectively; after 4 cys figures were 57% (A: median E cumulative dose=360 mg/sqm) and 60% (B: median E cumulative dose=480 mg/sqm). After 8 cys median LVEF in arm A declined to 50% (median E cumulative dose 720 mg/sqm) while no further reduction was detected in arm B after 4 cys of P. Six pts, each arm, developed asymptomatic 20% decline in LVEF (from baseline) after 4 cys of CT: at the end of treatment (8 courses) the same reduction was detected in 16 pts arm A and 9 pts arm B. During therapy 7 congestive heart failures (CHF) were observed all in arm A: one pt died because of CHF after 1 cy of CT; 3 pts were admitted after 8 cys (class IV New York Heart Association -cumulative dose of E=1080 mg/sqm-) and 3 pts required prolonged digitalo-diuretic therapy (class III NYHA -cumulative E dose 240, 360, 720 mg/sqm-).

Conclusions: This randomized study confirms that the risk of developing either a CHF or an asymptomatic impairment in cardiac function strongly correlates with the cumulative dose of E. In arm B the sequential administration of single-agent high dose of P does not influence cardiac functions.

333 Multicenter randomized phase III study evaluating epirubicin/cyclophosphamide (EC) versus epirubicin/docetaxel (ED) for first line treatment of metastatic breast cancer (MBC): an interim analysis of safety and efficacy.

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Background: This trial was initiated in 2001 and designed to accrue approx 120 patients (pts) per treatment arm to evaluate the safety and efficacy of EC vs ED in MBC. Treatment arms: A (E/C 90 mg/m^2/600 mg/m^2 q 3 weeks x 6 cycles) and B (E/D 75 mg/m^2/75 mg/m^2 q 3 weeks x 6 cycles). Primary endpoint: clinical response rate (RR = CR+PR). Secondary endpoints: time to progression (TTP), toxicity (tox) and overall survival (OS). Interim analysis was carried out to objectively assess tox and RR.
Patients and Methods: Key inclusion criteria: MBC, measurable disease (WHO criteria), and no hormonal treatment option. Adjuvant treatment and pretreatment for MBC (operation, hormonal, radiotherapy) was allowed. Karnofsky index (KI) > 70%. Minimum life expectancy was 12 weeks. Assessment was based on intent-to-treat analysis.
Results: At data cut-off, the study had accrued 189 pts (arm A: 89; arm B 100). For toxicity, 216 (A) and 222 (B) treatment cycles, and for efficacy 65 pts (31 A, 34 B), were evaluable. Pts were equally distributed between treatment arms by age (median 55/56yrs), KI (median 90% for each arm), adjuvant treatment and pretreatment for MBC, and hormonal status (pre/post menopausal). Key grade III/IV tox (% of all treatment cycles, armA/armB): leukopenia 48.6/51.8; platelets 2.8/1.0; febrile neutropenia 4.0/6.0; nausea 0.5/3.5; vomiting 1.0/3.0; and alopecia 20.8/19.4. Clinical response (armA/armB): CR+PR 12/10 pts; SD+PD 18/15 pts. There were 6 deaths (arm A: 1 tumor-related; arm B: 5, 1 treatment-related, 4 tumor-related).
Discussion: The emphasis of this interim analysis is on the assessment of safety and tolerability. Data from 438 treatment cycles suggest that ED and EC have similar safety profiles. The high rate of tumor-related deaths may be attributed to the low 12-week threshold for life expectancy allowed in this trial. With only 65 of 189 pts evaluable for clinical response, these data are still immature for definitive conclusions. More meaningful efficacy data will be available in December 2002.

334 Final results of gemcitabine (G) and epirubicin (E) phase II trial in metastatic breast cancer (MBC) patients (pts).

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Background: E and G are both active in breast cancer. The efficacy and toxicity of G+E were evaluated in pts with MBC in this phase I/II trial. Preliminary results were previously reported (Campone 2001, ASCO 20:1940). Final phase II data are presented here.
Patients and Methods: Thirty-nine female pts, chemonaive or relapsed >12 months after adjuvant chemotherapy, were enrolled. Eligibility criteria included: 18-70 yrs, stage IV MBC, WHO performance status 0-2, ≥1 bidimensionally measurable lesion, life expectancy ≥12 weeks, and adequate bone marrow, liver, and renal functions. Initially, 23 pts were treated with G 1500 mg/m^2 (d1,8) and E 90 mg/m^2 (d1) in 21-day cycles (group A). Cumulative hematologic toxicities resulted in several dose reductions of G in group A and the schedule was modified per Conte et al (1999, ASCO 18:449). Fifteen more pts were treated using G 1250 mg/m^2 d1,4, and E 90 mg/m^2 d1 in 21-day cycles (group B).
Results: Thirty-eight pts completed a median of 6 cycles. The dose intensity was 78.7% for G and 87.1% for E in group A, and 91.4% for G and 91.7% for E in group B. Of the 37 pts evaluable for response, there was 1 CR and 16 PR, for an overall response rate (ORR) of 45.9% (95% CI, 29.9% to 62%); 15 pts had SD and 2 progressed. The ORR was 31.8% in group A, and 66.7% in group B. For all pts, the median duration of response was 8.5 mos (95% CI, 7.7 to 11.4), the median time to progression was 8.4 mos (95% CI, 7.4 to 9.4), and the median time to treatment failure was 4.8 mos (95% CI, 3.9 to 8.1). WHO grade 4 toxicities in group A included neutropenia (16 pts, 69.6%), leukopenia (12 pts, 52.2%), thrombocytopenia (4 pts, 17.4%), and anemia (2 pts, 8.7%). Grade 4 toxicitites in group B were neutropenia (10 pts, 66.7%), leukopenia (2 pts, 13.3%), and thrombocytopenia (1 pt, 6.7%). Two pts in group A died while on study, 1 of shock (not related to treatment) and bronchopneumonia (probably related) and another of edema and heart failure (not related).
Conclusions: In group A, the regimen of G was amended from 1500 mg/m^2 on d1,8 to 1250 mg/m^2 on d1,4 due to a higher overall toxicity and 1 toxic death. The group B regimen, G 1250 mg/m^2 d1,4 and E 90 mg/m^2 d1 every 21 days, is better tolerated, may be more effective than the group A regimen, and should be investigated further.

335 Three-year results from a multicenter phase II study of capecitabine plus paclitaxel in metastatic breast cancer.

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Background: Capecitabine (Xeloda) is a novel orally administered fluropyrimidine that is converted into its active form of 5-FU through a three enzyme activation pathway including thymidine phosphorylase (TP). TP is present at higher levels in malignant tissues and is further upregulated by cytotoxic agents such as the taxanes. Pre-clinical studies have shown a synergistic antitumor effect when capecitabine was administered with paclitaxel. In clinical studies, the combination of capecitabine and docetaxel resulted in improved response rate, time to tumor progression, as well as prolonged survival, suggesting a potential synergy of capecitabine with the taxanes. **Methods:** This multicenter phase II study evaluated the efficacy and safety of the combination of capecitabine (825 mg/m2 twice daily d1-14, q3 weeks) and paclitaxel (175mg/m2 d1, q3w) as a first or second line treatment in women with metastatic breast cancer. **Results:** Forty-eight patients were enrolled, of whom 47 were evaluable for this analysis. One patient is currently receiving study treatment. Patient characteristics at baseline were median age 52 years (range 35-76 years) and median Karnofsky Performance Status 90 (range 70-100). Patients received a median of 7 treatment cycles (mean = 8.8, range 1-37). To date, 6 (13%) complete responses, and 18 (38%) partial responses have been observed, for an overall objective response rate of 51%. Thirteen (27%) patients experienced stable disease. Based on Kaplan-Meier estimates, median time to tumor response is 3.9 months, median time to progression is 10.5 months and median overall survival time is 20.6 months. The most common related grade 3/4 adverse reactions are neutropenia (n= 7, 15%), hand-foot syndrome (grade 4 not applicable) (n= 5, 11%), and alopecia (n= 6, 13%). **Conclusion:** This data indicates that the combination of capecitabine and paclitaxel is effective and safe in patients with metastatic breast cancer.

336 Extended phase I study of capecitabine in combination with vinorelbine in pretreated patients with metastatic breast cancer.

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The oral fluoropyrimidine-based prodrug capecitabine (C) and vinorelbine (V) have significant efficacy in patients (pts) with advanced breast cancer. The present study investigated the combination of C and V in pretreated pts with metastatic breast cancer to determine the dose limiting toxicities (DLT) and the maximum tolerated dose (MTD). Schedule and dose levels (DL): V was given i.v. on days 1, 8, 22 and 29; C was administered twice daily p.o. on days 1-14 and 22-35, with the next cycle starting on day 43. DL 1: V 25 mg/m2 and C 2x 1000 mg/m2 daily; DL 2: V 30 mg/m2 and C 2x 1000 mg/m2 daily; DL 3: V 30 mg/m2 and C 2x 1250 mg/m2 daily. Results: A total of 32 pts were enrolled, median age 52 years (30-69), PS 1 (0-1), sites involved: liver 18 pts, lung 14 pts, bone 12 pts, and lymph nodes/local relapses 5 pts. All pts were pretreated with anthracyclines and/or taxanes as first-line chemotherapy (CT) for metastatic disease or in an adjuvant setting (3 pts with high-dose CT). The MTD was reached at DL 2 with 4 DLT out of 6 evaluable pts (1 pt nausea/vomiting NCIC-CTC grade 3, 1 pt neutropenia grade 3 and prolonged treatment delay, 1 pt neutropenia grade 4, 1 pt with febrile neutropenia/diarrhea grade 4). To confirm the recommended dose in terms of safety, 18 pts were enrolled on DL 1 to a total of 24 pts. So far, 2 DLT out of 19 pts at the recommended dose (DL 1) were observed (2 pts with neutropenia grade 4, incl. 1 pt with febrile grade 3 infection). Thirteen out of 25 response evaluable pts showed a confirmed objective response (52%[95%CI:31-72%]). Conclusions: The combination of capecitabine and vinorelbine is feasible and shows a favourable toxic profile. The promising antitumor activity merits further clinical investigation.

337 Phase II study of vinorelbine, epirubicin and continuous 5-fluorouracil infusion as first line treatment in anthracycline naive metastatic breast cancer.

Berruti A, Bitossi R, Gorzegno G, Donadio M, Nigro C, Ardine M, Bertetto O, Bottini A, Danese S, Bertone E, Sarobba MG, Farris A, Bellino R, Katsaros D, Castiglione F, Grassi L, Marinone C, Dogliotti L. Azienda Ospedaliera San Luigi, Orbassano, Italy

A multicenter phase II trial has been conducted to test the activity of a combination regimen of vinorelbine (VNB), epirubicin (EPI) and 5-fluorouracil (5-FU) continuous infusion as first line approach in advanced breast cancer patients not previously submitted to anthracyclines in adjuvant setting. Up to March 31 2002, 51 consecutive patients entered the study. Demography was as follows: median age 58 yrs (range 30-75), median performance status 1 (0-3). Twenty-two patients (43%) had liver metastases, 23 (45%) lung, 26 (52%) bone, 13 (26%) soft-tissue and 4 (8%) other metastatic sites. Treatment consisted in VNB (25 mg/m2) on days 1 and 15 and epirubicin 35 mg/m2 on days 1,8,15, very 28 days. 5-fluorouracil was administered as continuous infusion at 200 mg/m2/daily without interruption. A total of 234 cycles have been up to now administered. On the whole the scheme was well tolerated: grade 3/4 leucopenia was observed in 30 cycles (12.8%) grade 2/3 anemia in 20 cycles (8.5%), grade 3 thrombocytopenia in 1 cycle (0.4%). Grade 2/3 stomatis was observed in 19 cycles (8.1%), grade 2 diarrhea in 3 cycles (1.3%), grade 2/3 nausea/vomiting in 21 cycles (8.9%). Asthenia grade 2/3 occurred in 18 cycles (7.7%) while neurotoxicity grade 1/2 in only 5 (2.1%). The activity of the regimen on the first consecutive 47 cases (4 cases are too early) was as follows: 3 patient (6.4%) attained a complete response (CR) according to the WHO criteria, and 23 (48.9%) a partial reponse (PR) for an overall response rate of (55.3%). Thirteen patients (27.7%) obtained a stable disease (SD), 5 (10.6%) progressive disease (PD) and 3 (6.4%) were not assessable, 2 of them for early interruption due to toxicity. These preliminary data suggest that this combination regimen is active and manageable. Final results of this trial will be provided at the meeting.

338 Vinorelbine (VR) and capecitabine (CAP) in metastatic breast cancer: Phase I/II study with correlative genotype, phenotype, and pharmacokinetics.

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Background: Past Phase I dose escalation studies with cancer therapeutics have used body surface area (BSA) dose escalation schemes despite evidence that chemotherapy clearance in many cases is not well correlated with BSA. This practice requires dose and BSA calculation, which can increase prescribing errors, and is difficult with oral formulations of drugs.

Methods and Results: We performed a Phase I/II study of capecitabine (CAP) (flat dose 3000 mg/d x 14 days q 3 wk) with dose escalation of vinorelbine (VR) (flat dosing, beginning at 20 mg IV days 1&8 q 3 wk) in patients with metastatic breast cancer. The dose escalation phase of the study has completed. 19 patients were enrolled between October 2000 and March 2002. The only non-hematologic Grade 4 toxicity was thromboembolism (2 patients). Grade 3 non-hematologic toxicity included peripheral neuropathy and elevations in hepatic aminotransferases (1 patient each). Grade 4 neutropenia was seen at the highest dose levels of vinorelbine (50 mg IV days 1 & 8), and febrile neutropenia was seen in 4/19 patients (all dose levels). The treatment was well tolerated overall. The MTD in this pretreated population was established as 40 mg IV of VR days 1&8 in combination with 3000 mg/d x 14 d CAP. 16/19 patients are evaluable for response, with 6/16 PR (37.5%) and 4/16 SD (clinical benefit = 62%). Pharmacokinetics of VR were performed on all patients in the study and results will be available in December. Area under the curve (AUC) of VR will be modeled against results of a phenotypic test for CYP3A4 metabolism, the erythromycin breath test, and other clinical factors. Analysis for genetic polymorphisms in CYP3A4 and other metabolizing enzymes will be performed. We plan to treat 10 patients at the MTD to obtain additional pharmacokinetic, pharmacodynamic, and pharmacogenetic data.

Conclusions: Flat dosing of drugs such as CAP and VR, whose clearance is poorly correlated with BSA, is feasible and convenient. This is an active and extremely well tolerated combination therapy in breast cancer which causes minimal alopecia. VR will soon be available in an oral formulation, and this regimen deserves attention as all-oral combination chemotherapy.

339 Vinorelbine and infusional 5-fluorouracil as first line chemotherapy in elderly patients with metastatic breast cancer : a phase II study.

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Background: Recent studies have demonstrated the satisfactory activity and the acceptable toxicity of Vinorelbine and 5-Fluorouracil in pretreated metastatic breast cancer : in this study we evaluated, in elderly patients (pts), the efficacy of a particular schedule of this combination chemotherapy which, theoretically, should offer a better profile of toxicity.

Materials and methods: From June 1999 to June 2001 we consecutively enrolled thirty aged pts with untreated metastatic breast cancer. Main characteristics: median age 74 (range 70-78); number of metastatic sites: one(9/31pts),two (15/31),three (5/31); visceral metastases were present in 21/31 patients. ECOG P.S.:1 (12/30) / 2(18/30).

The schedule of treatment was: Vinorelbine 25 mg/m2 i.v. D1 and D14 plus 5-Fluorouracil 250 mg/m2/day i.v. protracted infusion from D1 to D14 every 28 days. All patients received, at least, three cycles of this treatment. A total of 131 cycles of chemotherapy were administered.

Results: Response Rate was 50% (15/30) : no CRs were seen. SD:33% (10/30),PD 17% (5/30). Median time to progression and median survival were 19 weeks and 52 weeks, respectively. After 1-year follow-up 19 patients (63%) were still alive. Toxicity was generally mild: two patients (6%) developed G4 neutropenia without fever; G3 neutropenia 40 % (12/30), G3 anemia 20%(6/30), G3 thrombocytopenia 17%(5/30). G3 mucositis 17% (5/18). Two (6%) patients developed G2 hand-foot syndrome and other three patients showed grade 2 sensorial neuropathy. Nor sepsis neither any toxic death occurred.

Discussion: Elderly women with metastatic breast cancer are generally poor prognosis pts and the role of chemotherapy is merely palliative. This schedule of Vinorelbine and 5-Fluorouracil combination chemotherapy showed a substantial activity with a fully manageable profile of toxicity and it should be considered as valid and safe therapeutic option in this setting.

340 Vinorelbine and infusional 5-FU (VIF) - a phase II study in metastatic breast cancer.

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Background 5-fluorouracil (FU) given by continuous infusion (CI) has shown significant activity in MBC even in heavily pre-treated pts. It is well tolerated with manageable toxicity. Vinorelbine (VNR) has also shown substantial single agent activity in MBC and is well tolerated.

Method We report a study in progressive MBC of CI FU (200 mg/m2/day) plus VNR (30 mg/m2 i.v. day 1 and 8 each 21 days). CT continued to progression or 8 cycles. Response was assessed after each 2 cycles according to WHO criteria.

Patients: 61 pts have been registered, data is available on 59. All had previously had an anthracycline or anthracenedione (AN), 13 as adjuvant CT. 26 had received 2 lines of previous CT, 9 had 3 or more, 21 had a previous taxane (TX). Median age was 50 (28 - 78). ECOG performance score was 0 in 9 (15%), 1 in 33 (56%), 2 in 9 (15%), 3 in 2 (3%), unknown in 6 (10%).

Results: 259 cycles were evaluated for toxicity. Nausea or vomiting occurred in 32% (ECOG grade (g) 3-4 6%), diarrhoea in 12% (g3-4 1%), peripheral neuropathy in 12% (g 3-4 1%), constipation in 17% (g3 or g4 4%), palmar-plantar erythema in 9% (g3 or g4 in 3%), mucositis in 21% (g 3-4 3%). 12 pts were admitted with neutropenia or neutropenic sepsis. Treatment delay occurred in 29% of all courses particularly in the first 2 cycles. Median dose intensity (DI) for VNR was 15 mg/m2/wk (76% of protocol) and for 5FU was 135 mg/m2/day (67% of protocol). 10 pts had complications of venous access (Hickman or PICC line) and in 6 this lead to treatment being stopped. Response (CR or PR) was observed in 27 (46%) with 8 (14%) showing stable disease for more than 12 wks. Response was seen in 10 of 21 (48%) pts previously exposed to a TX

Conclusions: VNR plus CI FU has good activity in MBC even in pts previously treated with AN and TX. It is generally well tolerated but neutropenia and dose delay mean a DI of VNR of 15 mg/m2/wk is the maximum that can be achieved. 5FU at 200 mg/m2/day causes little toxicity but long term venous access remains a problem.

341 Oral Idarubicin and capecitabine: a dose-finding study confirms this as a safe, effective 1st line therapy for older women with breast cancer.

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Capecitabine has been established as a safe and effective oral therapy for advanced breast cancer, at least as efficacious as taxol or CMF. Idarubicin is an oral anthracycline with activity similar to single agent adriamycin in advanced breast cancer, but is less cardiotoxic. In combination with oral cyclophosphamide, it was found to be active but with significant neutropenia. Given that many patients would prefer to take oral chemotherapy, we conducted a dose finding study using standard phase I design, to find the MTD of the combination of idarubicin given on days 1 - 3 and capecitabine in divided doses on days 1 - 14 of a 21 day cycle.

Between June 1999 and June 2002, 14 women were treated aged between 55 & 75 years old (median 66), with chemotherapy-nieve locally advanced (2) or advanced breast cancer (12) (adjuvant CMF completed at least a year earlier was allowed). Normal cardiac function, and adequate renal and hepatic function were required. A median of 4 courses of treatment was given, and the Dose Limiting Toxicity of diarrhoea was defined at level 3.

No significant changes were seen in ejection fraction before and after therapy. These data confirm this oral combination chemotherapy regimen to be active and well tolerated, with less neutropenia than was seen with idarubicin/ cyclophosphamide regimens. Accrual continues at dose level 3a, as per protocol, since the DLT appears related to the capecitabine.

Dose Level	Idarubicin mg/m2/day	Capecitabine mg/m2/day	Maximum grade Diarrhoea	Maximum grade Neutropenia	Response
1	10	1500	4 (1 patient)	2	2/3 (3 Unassesable)
2	10	2000	1 (2 patients)	3	2/3
3	10	2500	3 (2 patients)	3	2/5
3a	12.5	2000	Only one	patient enrolled	to date

342 Phase II study of gemcitabine (G) and doxil (D) combination chemotherapy in patients with metastatic breast cancer: preliminary results.

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D and G are drugs with different toxicity profiles and mechanisms of actions that have both demonstrated anti-tumor activity in patients with metastatic breast cancer (MBC). A phase I study evaluating the safety of this combination in MBC patients has previously been reported (Rivera, et al JCO 19:1716-22, 2001). These results led to a phase II trial, further evaluating this combination. Interim results from this study are reported here. All patients had measurable disease, no prior chemotherapy for their metastatic disease, and a Zubrod < 2. Patients were allowed to have prior anthracycline-based adjuvant chemotherapy if their last dose of anthracycline was at least 12 months prior to initiation of treatment and there was no evidence of anthracycline-resistance. There was no limit on the prior cumulative dose of anthracycline received by the patients. The study is being performed within the MD Anderson CCOP sites. D was given at a starting dose of 24 mg/m2 iv over 2.5 hrs on Day 1 and G was given at a dose of 800 mg/m2 iv over 30 min. on Days 1, and 8, of each 21-day cycle. Forty-eight patients were entered in the study. Median age is 54 yrs (range, 33-75). Twenty-seven (56%) patients received prior adjuvant chemotherapy with 19 receiving prior anthracyclines, 11 received a taxane, and 10 received both. A total of 244 courses have been administered. No cardiac toxicity has been observed. The worst NCI grade toxicities observed follows: grade 3/4 granulocytopenia in 12 patients, grade 3 thrombocytopenia in 8, grade 3 anemia in 3, grade 3 vomiting in 2, grade 3 fatigue in 10, grade 3 stomatitis in 4, and grade 3 hand-foot syndrome in 2. There has been 1 episode of neutropenic fever. Two patients developed hemolytic-uremic syndrome more than a month after completing chemotherapy. Thirty-five patients are evaluable for response. Four patients were considered inevaluable and 9 patients were too early to evaluate. We have seen 2CR, 16PR, 8SD, and 9PD. Responses were also observed in patients whom had previously received anthracyclines. The study is still ongoing and an update of this data will be provided.

343 Higher trough marimastat levels are associated with accelerated disease progression and worse survival: results of a randomized phase III trial comparing marimastat with placebo in metastatic breast cancer (MBC) after first-line chemotherapy: an Eastern Cooperative Oncology Group trial (E2196).

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Background: The matrix metalloproteinases (MMPs) are a family of stromal enzymes involved in the metastatic cascade and angiogenesis. Marimastat (M) is an orally administered MMP inhibitor.

Methods: 180 eligible patients with MBC who had responding or stable disease after 6-8 cycles of first-line doxorubicin and/or taxane-based chemotherapy were randomized (2:1) to receive M 10 mg (N=115) or a placebo (P) (N=65) twice daily beginning after the completion of chemotherapy, continued until disease progression. Plasma was obtained at baseline and at 1, 3, 6, and 12 months for trough M and MMP-9 level.

Results: In comparing the M vs. P groups, there was no significant difference in median progression-free survival (PFS; 4.6 vs. 3.1 months; p=0.49) or overall survival (OS; 24.7 vs. 35.9 months; P = 0.173). Cox proportional hazard model indicated no significant effect of M on PFS or OS after adjusting for known prognostic factors. Higher trough plasma M level at month 1 or 3 were associated with a greater risk for progression and death (Table 1). Grade 2-3 musculoskeletal toxicity (MST) occurred more often with M, and was associated with a significantly worse OS (19.5 vs. 29.4 months; p=0.019) but not PFS when occurring during the first 3 months.

Conclusions: Marimastat (10 mg PO BID) did not significantly prolong PFS in this patient population (the primary endpoint). Higher plasma M levels were associated with a significantly greater risk of disease progression and death, and early M-associated MST was associated with worse survival.

Table 1. Influence of Maximum Trough M Level at Month 1 or 3 on PFS and OS

Trough M Level	No.	HR for Progression (P value)	HR for Death (P value)
>/= 10 ng/ml	69/115 (60%)	1.53 (0.03)	2.05 (0.03)
>/= 20 ng/ml	48/115(42%)	1.50 (0.051)	2.30 (0.02)
>/= 30 ng/ml	35/115(30%)	Not significant	5.05 (0.0003)
>/= 40 ng/ml	20/115 (17%)	Not significant	6.39 (0.005)

344 Inflammatory breast cancer (IBC): retrospective analysis of 74 patients treated in a single institution.

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Background: IBC represents a rare but lethal form of breast cancer. Despite a multidisciplinary approach, the global survival range 30-50% at five years. We analysed 74 pts with non metastatic IBC treated in a single institution.The clinical, biological and therapeutic factors associated with survival were recorded.

Patients and Methods: Median age was 50.57 years (range, 24 to 81 years). All pts had IBC defined as the T4d of the UICC classification.Histology was ductal carcinoma in 87.32%, grade SBR III in 81.1%, and both negative hormonal receptors (HR) in 47.83%. Dermal lymphatic involvement was present in 78.26%. An immunohistochemistry essay was performed to evaluate expression of p53, HER2, EGFR and E-cadherine. All pts had a primary chemotherapy including anthracyclines except for 4 pts and high-dose chemotherapy (HDC) with stem cell transplantation for 54 pts. Mastectomy was performed for 62 pts after induction chemotherapy and completed by radiotherapy for 70 pts. Tamoxifen was prescribed to pts with positive HR.

Results: The median duration of follow-up from diagnosis was 48.5 months (range, 9 to 170 months). At 60 months, the Kaplan-Meier estimates of PFS and OS from diagnosis were 24.33% and 41.2%, respectively. In monovariate analysis, positive HR and presence of HDC were associated with better PFS (p=0.03;p=0.006) and OS (p=0.009;p=0.001). In multivariate analysis, positive HR and HDC remained significantly associated with PFS (RR=1.85; RR=2.12) and OS (RR=2.12;RR=2.28)

Discussion: This retrospective analyse shows a statistically significant improvement in terms of both PFS and OS observed in pts treated with HDC for IBC, but failed to provide the prognostic role of biological parameters, particularly expression of HER2, as well as pathologic response post-chemotherapy.

345 Five-year follow-up of a randomized clinical trial comparing high dose FEC supported by rHuG-CSF versus conventional FEC in the unilateral inflammatory breast cancer.

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IBC is a rare form characterized by a rapid progression and poor prognosis. Neoadjuvant CT had significantly improved the outcome of this disease. We conducted between 09/ 1993 and 12/ 1997, a multicenter randomized trial to compare the OS, DFS and efficacy (clinical and histological response) of HD-FEC to FEC75 in women with non metastatic unilateral IBC. One hundred fifty four pts with no prior CT were randomized to receive 4 cy of induction CT /21 days: HD-FEC: 5FU 750mg/m^2/d, D 1 to 4; epirubicin 35mg/m^2/d, D 2 to 4; cyclo-phosphamide 400 mg/m^2/d, D 2 to 4 + rHuG-CSF150 mg/m^2/d D 6 to 15)(Arm A, 77 pts). FEC 75: 5FU 500 mg/m^2 D1; epirubicin 75 mg/m^2 D1; cyclophosphamide 500 mg/m^2 D1 (Arm B, 77 pts). Responders to this induction CT entered a locoregional therapy (radio-therapy (RT) +/- surgery) followed by 4 cy of adjuvant FEC 75 every 28 days. The median age of pts was 47.5 yrs (26-66 yr). A total of 151 pts were evaluable for response. The ORR (CR+PR) was 83.1% in HD-CT and 66.2% in the FEC 75 arm (p=0.016), CR was 14.3% and 11.7% respectively.One hundred twenty two pts underwent surgery after 4 cy of induction CT (60 in HD-FEC and 62 in FEC 75) with an overall good histological response (complete sterilization and in situ lesions) of 16.7% and 6.4% in HD-FEC and FEC 75 resp. Breast conserving surgery was in 6.7% in HD-FEC arm and 4.8% in FEC 75 arm. 63 in each arm underwent RT and 120 pts received adjuvant CT (59 in HD-FEC and 61 in FEC 75). At a median follow-up of 5 yrs, a significantly longer DFS (p=0.01) and a significantly better OS (p=0.05) were observed in the HD-FEC arm. We conclude that HD-FEC+rHuG-CSF in first line therapy of IBC induced an improved RR (83.1% vs 66.2%, p=0.016), an improved recurrence free survival (p=0.01) and an improved survival (p=0.05).

346 Intraoperative magnetic resonance imaging in combination with lumpectomy grossing improves rate of negative surgical margins for invasive breast cancers.

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Background: For patients diagnosed with an invasive breast cancer by core needle biopsy, open magnet contrast enhanced Breast MRI allows for intraoperative post-excision evaluation of the surgical bed to assess for residual tumor. We evaluated the utility of intra-operative post-tumor excision (EX) MR contrast enhancement in conjunction with gross pathology (GP) margin assessment.

Material and Methods: 20 patients with T1 (n=20) and T2 (n=2) breast cancers underwent MR-guided EX in an open configuration 0.5 Tesla MR imaging system. Images were obtained prior to and following tumor EX. MR enhancing areas suggestive of residual tumor were resected. Each specimen was surgically grossed. Additional margins were excised based on the GP. Post resection MR images (PR-MRI) and GP margins were compared with each other, and with permanent pathologic (PP) margins, which served as the gold standard.

Results: Concordance between GP, PP, and PR-MRI was seen in 6 patients: all 3 margin assessments were positive (+) in 4 and negative (neg) in 2. In an additional 3 patients, the GP and PR-MR images were concordant with each other (+=1, neg =2), but discordant with the margins on the PP.

There was discordance between the GP margins and the PR-MRI margins in 11 patients. In 5, no enhancing area was seen on PR-MR images, and both the GP and PP margins were + (false-neg (FN) PR-MRI, true + (TP) GP): intra-operative shave margins performed based on the GP were neg in all 5 cases. In 4 patients, the GP margins were reported as neg, with the PR-MRI images and PP margins + (FN GP, TP PR-MRI): intra-operative shave margins performed based on the + PR-MRI images were + for cancer in 2 patients and neg in 2. In 1 patient, re-excision was prompted by a false + (FP) PR-MRI, with the GP and PP margins being negative (FP PR-MRI, TN GP): the shave margin prompted by the MR image was neg. Conversely, in 1 patient, a re-excision was prompted by a + GP margin, with the PR-MRI and PP neg (FP GP, TN PR-MRI): the shave margin was negative for residual tumor.

Conclusion: MR-guided lumpectomy utilizing an open configuration MR system obviated the need for a second surgery in 4 of 20 (20%) patients. 5 of 20 (25%) patients would benefit from ongoing MR imaging improvements.

347 Intraoperative ultrasound simplifies surgery for early breast cancer.

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Introduction: Almost half of recently diagnosed breast cancers are non-palpable. Mammogram directed wire localization for non-palpable lesions requires time and coordination by the surgeon and some discomfort for the patient. About 40% of these non-palpable lesions can be visualized by ultrasound. Use of intraoperative ultrasound may streamline the process of image guided surgery. Methods: We prospectively visualized 91 consecutive patients with non-palpable breast cancer between 1/98 and 5/02. After preoperative evaluation, often including needle biopsy, we performed ultrasound localization in the operating room immediately prior to definitive surgery. Breast cancers were localized using either blue dye or a guide wire. Sentinel node sampling was performed in appropriate patients. Frozen sections were not routinely performed. Tumor data and re-excision rates were noted on all patients. Results: Ultrasound correctly localized all lesions at surgery. Negative margins for invasive carcinoma were found in 95% (86/ 91) of patients. Re-excisions were performed in only 8% (7/91) of patients. Including both in-situ and invasive disease, negative margins were found in 90% (82/91) of patients. Positive margins were due to the presence of non-calcified ductal carcinoma in-situ (44%) or multifocal invasive disease (56%) noted at final histology. Mastectomy was necessary in 12% of patients, usually due to extensive DCIS. Conclusions: Use of ultrasound has facilitated the localization breast cancer in the operating room. This improves the process of image guided surgery for both patient and surgeon. Ultrasound localization is accurate, time efficient, technically feasible and easier for the patient. Re-excision rate is very low and similar to mammographic localization. The presence of extensive in-situ disease identifies patients at high risk for positive margins. Intraoperative ultrasound localization should be considered whenever a non-palpable breast cancer requires excision.

348 Nipple sparing mastectomy: an alternative to breast conservation?

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Introduction: Resection of the nipple areola complex (NAC) is considered standard practice as part of a mastectomy. Preservation of the NAC has been reported previously, however, it is not practiced commonly. The purpose of this study was to develop experience with this approach and to evaluate cosmetic outcome.

Methods: NAC preservation was performed in 34 skin sparing mastectomies in 30 patients. Mastectomies were performed by conserving the skin envelope through incisions planned preoperatively by the oncologic and plastic surgical teams. Tissue beneath the NAC was removed completely and sent for frozen section. The NAC was removed if there was evidence of cancer on frozen section. Patients were not candidates for NAC preservation if their tumors were less than 3 cm from the NAC.

Results: 25 nipple-sparing mastectomies were performed for breast cancer treatment and 9 for prevention. Among the breast cancer group, 20 had invasive cancer and 5 had DCIS. Immediate breast reconstruction (13 TRAM flaps and 21 implants) was performed in all cases. Frozen section of the nipple core tissue found cancer in 3 cases leading to nipple resection. Complete viability of the NAC was achieved in 87% of cases (27 of 31) with partial loss in 13% (4 of 31). Medially placed incisions appeared to compromise viability of the NAC. Superficial tissue loss was common, resolving within several weeks, without compromising the cosmetic outcome. NAC viability was not dependent upon the type of immediate reconstruction. Initial cosmetic outcomes were excellent in the majority of patients.

Conclusion: Nipple sparing mastectomy with immediate reconstruction is an important option for patients with early stage breast cancer and for patients choosing prophylactic mastectomy. Medial incisions may result in full or partial loss of the NAC and should be avoided. Cosmetic outcome of this approach appears to rival that of breast conservation and may provide an important alternative.

349 Utility of imaging techniques in surgical treatment and diagnosis of breast carcinoma in women presenting with nipple discharge.

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Background: There is no consensus regarding the use of the various diagnostic tests and surgical procedures available to confirm or rule out breast cancer in patients presenting with nipple discharge. This study was designed to identify patient characteristics associated with the diagnosis of breast cancer and to determine the utility of imaging techniques in surgical treatment of patients presenting with pathologic nipple discharge. Methods: Medical records of 94 patients who underwent a biopsy procedure for histologic diagnosis and/or treatment at our institution between August 1993 and September 2000 were reviewed. Results: Nineteen cancers, 62 papillomas, and 13 other benign lesions were identified. Logistic regression analysis revealed mammographic (RR=10.47, 95% CI 2.36 to 46.39, p = 0.0002) and sonographic (RR=5.54, 95% CI 1.27 to 25.40, p = 0.028) abnormalities as independent factors associated with a malignant diagnosis. The sensitivity and specificity of mammography and sonography in the detection of cancer were similar (68.4% versus 80% and 75.7% versus 61.2%, respectively). Cytology had a low sensitivity (26.7%) despite its high specificity (81.1%) and negative predictive value (79.6%). Ductography had a very low specificity (5.6%) despite its high sensitivity (100%) and negative predictive value (100%). In three patients with cancer (15.8%) and thirty patients with a papilloma (48.4%), ductography was the only means of detecting lesions to be resected. Patients who underwent ductography-guided surgery (n = 42, 50%) or any surgical procedure including a localization study (n = 66, 78.6%) were significantly more likely than patients who underwent central duct excision alone to have a specific underlying lesion identified (p=0.045 and p=0.033, respectively).Conclusions: Abnormalities on mammography and sonography in patients with nipple discharge should alert physicians to the possibility of a breast cancer diagnosis. In patients with pathologic discharge with normal findings on physical examination and other imaging studies, ductography may be the only means of localizing and resecting breast lesions associated with nipple discharge.

350 Management of Tis-T1 breast cancer using radioguided surgery with sentinel lymph node biopsy in day-surgery regimen.

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Background: The Sentinel Lymph Node Biopsy (SLNB) with radioguided surgery (RGS) can often be performed in an outpatients setting with local anesthesia with paravertebral block C7-T7.

Material and Methods: 105 patients (pts), average age 54,5 years (range:33-75ys), underwent a breast cancer excision and sentinel lymph node biopsy using preoperative lymphoscintigraphy (two bilateral procedures). All patients were scheduled to undergo surgery on an ambulatory basis. An informed consent was taken for all pts. The technique involves a intra and peritumor injecton of an average of 99-Tc. The surgical procedure of the tumor and axillary SLNB included margin of more than 1 cm of normal tissue. Out of 105 cases of breast lesions, 57 invasive carcinomas staged as pT1a were identified; in the remaining 48 cases resulted to be DCIS, 7 of them with microinvasion (4 with microinvasion of 1 mm; 3 cases more than 1 mm).

Results: The surgical time averaged 75 minutes for RGS with SLNB. There were no intraoperative complications. Pathologic analysis revealed in all excisions specimens. The primary breast lesion was located and excised in all cases (identification rate 100%). 94 of 105 pts rated the overall surgical, anesthetic and recovery experience as "very satisfactory". Pts typically expressed pleasure at the ability to return home and stressed the ease of recovery.

Discussion: Our results indicated that Sentinel Lymph Node Biopsy and Radioguided Surgery associated with troncular or paravertebral block are a significant step forward in the search for less aggressive treatments for early breast cancer.

351 Misleading residual breast lesions secondary to cryosurgery: results at two years follow up.

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Non-surgical techniques such as cryosurgery (CS) and radiofrequency for ablating tumors are being studied as alternatives to surgical excision. Preliminary data indicate that tumors can be destroyed, but a series of problems still remain. High costs, good cosmetic results restricted only to non-superficial lesions, limited pathological analysis including lack of surgical margins evaluation, together with difficulty in accurately assessing breast imaging after treatment are major drawbacks of these techniques. The purpose of this study was to demonstrate the difficulties related to clinical, imaging and histological findings of cryotreated benign breast lesions.

Material and Methods: A total of 39 patients were treated with a cryosurgical system (Sanarus Medical Inc.,Pleasanton,CA) guided by ultrasound. The first 11 patients had elective reduction mammoplasty 3 to 6 months after CS. The remaining 28 had core biopsy-proven fibroadenomas and were followed by clinical examination, mammography and ultrasound for at least 2 years post CS.

Results: CS of normal breast tissue in 11 patients showed extensive cryolesions characterized by fat necrosis and organizing inflammation seen until 6 months post-therapy. Histological examination of excised cryotreated fibroadenomas demonstrated epithelial lining degeneration and well preserved stroma. Overall, there was stable tumor volume reduction up to 90% at 24 months in fibroadenoma patients measured by ultrasound. However, 80% of the cases presented abnormal breast ultrasound, 38% distorted suspicious mammographic findings and the majority of the patients had diffuse palpable masses.

Conclusion: Although cryoablation was efficient in reducing tumor volume, there were misleading residual abnormalities which may limit therapeutic benefit of the technique and its potencial use in breast cancer.

352 Surgical procedures after preoperative chemotherapy (PCT) in patients (P) with primary operable breast cancer (BC) in the multicenter geparduo-trial.

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PCT in operable BC raises increasing attention as the individual effect of systemic treatment can be monitored online and the rate of breast conservations (BCT) can be increased significantly. However, there is only limited information on surgical procedures in multi-institutional treated cohorts of P.

Methods: In total 913 P with operable stage T2 - T3 BC were treated in the GEPRADUO-trial with either 4 cycles of Adriamycin / Docetaxel or 4 cycles Adriamycin / Doxorubicin followed by 4 cycles of Docetaxel (von Minckwitz et al. Proc. ASCO 2002). So far 488 reports from 55 institutions on surgical procedures were analysed and correlated with clinical parameters and histomorphological features.

Results: BCT was possible in 79% of P. Tumorectomy, segmentectomy, or quadrantectomy was performed in 29%, 30% and 20%. Modified radical mastectomy was necessary in 21%. Breast reconstructions were performed using Latissmus Dorsi Flap in 29 P, TRAM flap by 2 P, tissue expander in 13 P or primary implants in 8 P. Whereas median tumor size for all groups of surgical procedures was between 3.9 and 4.4 cm before chemotherapy, median tumor diameter before surgery was greatest before mastectomy (3.3 cm) and smallest before tumorectomy (2.0 cm). All P with a clinical CR had BCT whereas the rate decreased to 72% for PR, 64% for NC and 36% for PD. A second operation due to involved / close surgical margins was performed in 15%. Histological examination revealed in 16% further invasive tumor, in 30% in-situ tumor residuals only and in 54% no remaining tumor. In case of pre-surgical needle localization or intra-operative biopsy radiogram the rate of second operations were higher (19.7% and 30.0%), whereas in case of intra-operative frozen sections the rate was lower (8.4%).

Conclusion: Participating surgeons have adapted the size of breast biopsy to the tumor size after PCT resulting in a high rate of BCT. The best predictor for BCT was a clinical CR.

353 Endoscopic breast surgery for breast cancer as a skin sparing operation.

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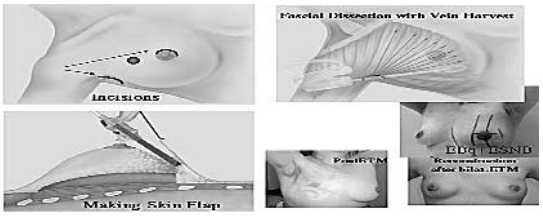
Since 1995, endoscopic surgery for breast cancer have been performed at our center to minimize skin incision and spare skin. Through small areolar and axillar skin incisions, dissecting tissues with vein harvester and visitorocher were accomplished to make a space under skin flap and between a pectral major muscle & a fascia. Under endoscopic visionand inn an air pouch, hemostat and resection of the remnant tissue surrounding proposed mastectomized area was carried out with ultracision. Axillar LND dissection was made through the axillar incision under direct vision. Skin sparing endoscopic total mastectomy (ETM) was carried out in 140 patients and half of patients among the cases with ETM had endoscopic reconstruction, using latissimus dorsi muscle & fat flap or saline filled silicone bag. Endoscopic partial mastectomy was done in 145 patients Average blood loss of this surgery was less than that of conventional mastectomy. And simultaneous reconstruction of skin spared breast resulted in excellent postoperative cosmetic outcome. Meticulous pathological examination and postoperative follow-up CT failed to find remnant breast tissue or cancer. Two locally recurrent patients were experienced. We report our procedures and excellent result of endosopic breast surgery for cancer.



354 Computer simulating volume rendering breast operation model.

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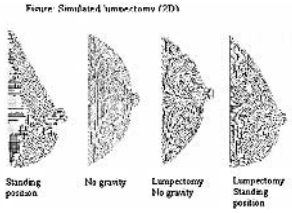
Background: Breast conservation surgery is one of the standard operation in breast cancer patients. For better clinical outcome two important contradictory factors have to be satisfied, such as tumor control and cosmetic appearance. Furthermore surgical design must be decided by the operating surgeon's skill and experiences. If post-operative deformations can be simulated pre-operatively, it could provide benefits to the design of operation. The purpose of this study is to establish volume rendering human breast model and develop it a virtual breast operation model. **Materials and Methods:** The material used in this breast model was hypothesized as a single-component. From the data based on the deformations of breast at various positions, parameters (Mass density, Shear modulus, Poisson's ratio) of normal breast were gathered. The simulated breast is represented in ***LS-DYNA*** which is originally developed for the 3-dimensional finite element structural analysis and can analyze the system using a series of model with fluid-solid interaction. Using this program volume rendering breast operations are reproduced. **Results :** This simulating breast model can not only represent actual breast deformation, but also simulate a breast deformation such as volume reduction and enlargement. (Fig). **Discussion:** Recent progress of imaging diagnosis can reveal precise extension of breast cancer pre-operatively. But at present these data are used only for diagnosis. Utilizing these digital data the virtual breast operation model could simulate a breast conservation and enlargement surgery. Moreover since this program may predict post-operative appearance, surgeon can utilize it to choose a preferable surgical design pre-operatively and may help patients for the better understanding of the treatments.



355 Zoledronic acid (4mg) significantly reduces the relative risk of developing a skeletal-related event compared with pamidronate (90mg) in patients with breast cancer and bone metastasis.

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We previously reported the equivalence of zoledronic acid (Zol) 4mg and pamidronate (Pam) 90mg in preventing skeletal-related events (SREs) in a 13-mo, phase III trial in breast cancer patients (N=1130) with bone metastases (Rosen LS, et al. *Cancer J*. 2001;7:377-387). Patients were randomized to receive Zol 4 or 8mg via 15-min infusion or Pam 90mg via 2-hr infusion, every 3 to 4 wk, plus appropriate antineoplastic therapy. We report here the 25-mo follow-up data. 412 patients completed the original 13-mo study and continued to receive study medication for 1 additional year. The Zol 8mg dose was associated with serum creatinine elevation, offered no increased efficacy, and was subsequently decreased to 4mg (Zol 8/4mg group). The incidence of SREs (pathologic fracture, spinal cord compression, surgery to bone, or radiation therapy to bone) was evaluated at 25 mo. The proportion of patients experiencing at least 1 SRE (study-defined primary endpoint) was 46%, 48%, and 50% for Zol 4mg, Zol 8/4mg, and Pam 90mg, respectively (*P*=.340, Zol 4mg vs Pam). Similarly, median time to first SRE was 377, 367, and 370 days for Zol 4mg, Zol 8/4mg, and Pam 90mg, respectively (*P*=.189, Zol 4mg vs Pam). However, the preplanned analysis of multiple SREs via the Andersen-Gill method demonstrated a benefit for Zol 4mg over Pam 90mg. Andersen-Gill multiple event analysis accounts for the time to first and subsequent SREs, and revealed that the risk of developing an SRE was significantly lower in the Zol 4mg group (0.816; 95% CI=0.671, 0.993) compared with the Pam 90mg group (*P*=.042). A similar decrease was noted in the Zol 8/4mg group (0.828; 95% CI=0.680, 1.009) compared with Pam 90mg (*P*=.061). In summary, Andersen-Gill multiple event analysis is a rigorous and sensitive method to account for all clinically relevant SREs that may not be adequately evaluated via traditional analyses (ie, proportions and time to first SRE). Based on long-term follow-up (25 mo), these findings support the conclusion that breast cancer patients receiving Zol 4mg via 15-min infusion are at significantly lower risk of developing an SRE than are patients receiving Pam 90mg via 2-hr infusion.

356 The bisphosphonate zoledronic acid downregulates signalling through Ras effector pathways in human breast cancer cell lines.

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Background: Bisphosphonates are of proven efficacy in limiting skeletal related morbidity in patients with advanced breast cancer. Their role in adjuvant therapy remains to be determined and the mechanisms by which they exert their effects are inadequately understood. We have previously demonstrated that bisphosphonates directly induce apoptosis in breast cancer cell lines through impaired membrane localisation of Ras and that zoledronic acid impairs adhesion of viable breast cancer cells to mineralised matrices prior to inducing apoptosis in these cells. We have now further investigated the signalling pathways involved in these effects.

Materials and Methods: MCF-7, T47 D and Hs578T breast cancer cells were treated with 100 µM zoledronic acid or vehicle for 1-3 days. Equivalent protein extracts from whole cell lysates were analysed by western blotting. Levels of phosphorylated and total AKT (protein kinase B), ERK (p44/42 mitogen-activated protein kinase), CREB (cyclic AMP response element binding protein) and bad[112] were measured and compared.

Results: Zoledronic acid affected ras mediated survival pathways in a differential and cell-specific manner. Treated MCF-7 and T47 D cells demonstrated a downregulation of phosphorylated ERK but not phosphorylated AKT. Levels of phosphorylated CREB and bad[112], signalling proteins downstream of ERK, were also reduced in both cell lines. Conversely, treated Hs578T cells demonstrated marked downregulation of phosphorylated AKT levels and a downregulation in levels of phosphorylated bad[112] but no demonstrable change in ERK or CREB levels.

Discussion: Our findings suggest that zoledronic acid downregulates effectors of the Ras pathway in a cell specific manner. ERK appears to be a more important regulator of survival than AKT in MCF-7 and T47 D cells although the converse appears to be the case in Hs578T cells. The factors that determine the dominant survival pathway in individual cell types and the regulators of this process remain to be determined.

Alteration in levels of activated signal transduction protein with zoledronic acid treatment.in breast cancer cell lines

	AKT	ERK	CREB	bad[112]
MCF-7	→	↓	↓	↓
T47 D	→	↓	↓	↓
Hs 578T	↓	→	→	↓

357 A Phase II study of ZD1839 ('Iressa') in tamoxifen-resistant ER-positive and endocrine-insensitive (ER-negative) breast cancer.

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The epidermal growth factor receptor (EGFR) pathway is associated with oestrogen receptor (ER)-negative phenotype and de-novo hormone resistance. Experimental evidence also suggests that for ER-positive breast cancer cell lines with acquired resistance to tamoxifen this may be in part mediated through increased signalling of EGFR pathways.

Twenty-two patients, with either ER-negative breast cancer (n=16) or ER-positive breast cancer that became clinically resistant to tamoxifen (n=6) were treated with ZD1839 500 mg po. The median age was 61 yrs (range 32-85 yrs). The metastatic sites of disease were lung (n=2), skin (n=2), pleura (n=3), liver (n=4), local regional disease (n=4), bone and metastatic lymphadenopathy (n=7).

At Visit 1 (4 weeks) 2 patients (9%) had a partial response, 10 patients (46%) had stable disease and 5 patients (23%) had progressive disease. Of the partial responders one had liver metastasis from an ER-positive tumour while the other had supraclavicular lymph adenopathy from an ER-negative cancer. Three patients had withdrawn from the study prior to Visit 1 due to side-effects. The remaining patients are pre-Visit 1.

Thus far one patient with stable disease has been treated beyond 6 months. Nine patients have been withdrawn due to disease progression. The median time to progression was two months (range 1-9 months). Two patients have died unexpectedly: one from bronchopneumonia and one from a cardio-respiratory arrest. Both were well at their last study visit. Side-effects thought to be attributable to ZD1839 were experienced by 59% of patients. Eight patients (36%) had a significant facial rash and 4 patients (18%) had nausea, vomiting and disturbance of bowel habit. Eight patients (36%) had a dose interruption followed by a dose reduction.

Early data suggest that ZD1839 may be effective in both ER-negative and tamoxifen-resistant ER-positive breast cancer.

'Iressa' is a trademark of the AstraZeneca group of companies.

358 Apoptosis and growth arrest of human breast cancer cells: the effects of CI-1033, a type I receptor tyrosine kinase inhibitor.

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Background: Transmembrane type I receptor tyrosine kinases EGFR and erbB2 have been extensively studied because they are over expressed in many cancers, including breast cancer. This over expression has been correlated with a more aggressive tumor phenotype. The orally active tyrosine kinase inhibitor CI-1033 is an irreversible inhibitor of Type I receptor tyrosine kinases. We evaluated the effects of CI-1033 on four human breast cancer cell lines, the SUM149 and SUM229 cells, which over express EGFR, and the SUM190 and SUM225 cells, both of which highly over express activated erbB-2. The SUM225 cells over express erbB-2 to a higher-level then SUM190 cells, and also express EGFR.

Materials and Methods: Cells were cultured under low serum conditions, harvested, and protein analyzed using 7.5% SDS-PAGE. For growth curve assays, cell nuclei were isolated and fixed in formalin, then counted using a Coulter Counter. BrdU exposed cells were fixed in 70% alcohol, and after staining with anti-BrdU antibody and Propidium Iodide, cells were analyzed with flow cytometry.

Results: Phosphotyrosine Western Blot analysis revealed that daily dosing with 0.1-1uM CI-1033 resulted in at least partial inactivation of tyrosine kinase activity of erbB-2 and EGFR in all four cells lines. With repeated daily dosing there was minimal return of tyrosine kinase activity in SUM190 cells after 24hrs of washout of the drug. There was no return of tyrosine kinase activity in SUM225 cells even 48hrs after washout of the drug. In contrast, in any of the cell lines, one dose of drug only partially inactivated tyrosine kinase activity, even with doses that resulted in non-specific cellular toxicity. Growth Curves confirmed growth stasis with daily dosing with 1uM, 0.1uM, 3uM, and 5uM in SUM190, SUM225, SUM149, and SUM229 cells, respectively. Growth curves at twice a day dosing of drug showed growth stasis of SUM190 cells at 0.1uM CI-1033. Flow cytometry revealed G1 arrest of SUM190 cells after 48 hrs of treatment with 1uM CI-1033, and apoptosis after 72 hrs of exposure. SUM225 cells showed apoptosis after 24 hrs of exposure to 0.1uM CI-1033.

Discussion: This novel tyrosine kinase inhibitor, CI-1033, shows promise as an anti-cancer agent by causing cell cycle arrest and apoptosis in human breast cancer cell lines. These results suggest that those tumors with higher over expression of activated erbB-2 will respond to lower levels of this inhibitor, and to shorter exposure periods. These results also suggest that tumors with over expression of EGFR are more likely to respond with growth stasis, then apoptosis, having implications for the adjuvant use of this drug. Chronic low dosing of CI-1033 will likely be the most effective, and least toxic, method of administering the drug.

359 Lack of effect of tamoxifen (T) on bone loss in pre and peri-menopausal women with breast cancer rendered postmenopausal after adjuvant chemotherapy (C): results of the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) adjuvant breast study MA12, bone add-on study.

Tonkin KS, Jackson S, Bramwell VHC, Hodsman A, Shepherd L, Pritchard KE. Cross Cancer Institute, Edmonton, AB, Canada; London Regional Cancer Centre, London, ON, Canada; St Josephs Health Centre, London, ON, Canada; NCIC-CTG, Kingston, ON, Canada; Toronto-Sunnybrook Cancer Centre, ON, Canada

Background: The randomized double blind NCIC-CTG study MA12 was designed to test benefit, if any, of (T) in women rendered postmenopausal 9-15 months after (C) (AC x 4, CMF or CEF x 6) in pre/peri-menopausal high risk node negative and node positive women. Methods: Bone density of spine (L2-L4) and hip were measured using dual energy x-ray absorptiometry (DEXA). Instruments from different manufacturers were used, but results are expressed as percentage change per year over study period, and are independent of machine type. Results: Values are compared to reference population, not influenced by use of bisphosphonates, estrogen, chemotherapy, or (T). Normative data was derived from Canadian Multicentre Osteoporosis Study (CaMos), a large ongoing population-based epidemiological, prospective osteoporosis study. CaMos involves N=9423, (2878 male and 6545 female) and samples individuals within 50 km of a DEXA machine. Mean age of MA12 women was 46.5 (range 36-54 with 6 under 40). At baseline, 14 women had osteopenia and 1 osteoporosis using T scores. Patients receiving placebo (n=17) exhibited a 1.2% per year loss in BMD (spine and hip). This is not different to patients receiving T (n=10), showing 1.2% per year loss (spine) and 1.1% loss per year (hip) comparing baseline to years 1,2, and 5. Compared to the age matched CaMos population data, the rate of loss of BMD is more than double the 0.5% per year measured. Full data on 40 women will be presented. Discussion: Although a relatively small sample size there appears to be no beneficial effect from T in this chemotherapy induced postmenopausal population.

360 Omega-3 fatty acids restore tamoxifen sensitivity in breast cancer cells with high Akt activity.

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Tamoxifen resistance is the underlying cause of treatment failure in many breast cancer patients. In hormone-resistant disease, the dominant influences on tumor cell growth are growth factors acting through specific receptor tyrosine kinases. Much of this signaling is mediated through activation of the Akt kinase, a downstream target of the PI3 kinase. Breast cancers with heightened Akt activity are frequently associated with an aggressive disease and resistance to chemo- and hormone-therapy induced apoptosis. Inhibition of PI3 kinase restores apoptotic response to tamoxifen in hyperactive Akt cells. Therefore agents which demonstrate Akt inhibitory properties are attractive therapeutic agents for the treatment of hormone-resistant breast cancer. Omega-3 fatty acids have proven to be potent and efficacious broad-spectrum protein kinase inhibitors. In this study we demonstrate that the omega-3 fatty acid, eicosapentaenoic acid, inhibits the kinase activity of Akt. Co-treatment with eicosapentaenoic acid renders breast cancer cells that overexpress a constitutively active Akt more responsive to the growth inhibitory effects of tamoxifen. These findings suggest that eicosapentaenoic acid may be useful for the treatment of tamoxifen resistant breast cancer cells with high levels of activated Akt. These observations have important clinical implications because they suggest that nutrient supplements have potential efficacy in specific treatment strategies for patients with breast cancer on the basis of specific molecular anomalies, and provide clinicians with potential therapeutic strategy in tamoxifen-resistant breast carcinoma.

361 2-deoxy-D-glucose potentiates the cytotoxic effects of ^{64}Cu-PTSM in a mouse model of breast cancer.

Aft RL, Lewis JS, Zhang F, Kim J, Welch MJ. Washington University Siteman Cancer Center, St. Louis, MO

Background: We have previously demonstrated that 2-deoxy-D-glucose (2-DG) treatment of breast cancer cell causes apoptosis and potentiates the cytotoxic effect of ionizing radiation and chemotherapuetic agents *in vitro*. We hypothesize that 2-DG acts by impairing the ability of cancer cells to repair damage caused by these agents through interruption of energy metabolism. We have now tested 2-DG in a mouse model of breast cancer alone and with ^{64}Cu-pyruvaldehyde-bis(N^4-methylthiosemicarbzone) (PTSM). ^{64}Cu-PTSM has been used as an agent for administering high doses of local radiotherapy after uptake by cancer cells (Lewis et al. Cancer Res 6:445,2002).

Materials and Methods: EMT-6 mouse breast cancer cells were implanted subcutaneuosly into the hind flanks of BALB/c mice. 5-8 mice were randomized to each treatment group. Tumor measurements were obtained twice weekly in two dimensions.

Results: 2-DG delivery was measured using FDG-microPET. 2-DG injected either IV or IP resulted in equivalent whole body distribution of FDG while PO or SQ administration exhibited less systemic uptake. No adverse reactions were observed in mice injected with up to 2 mg/gm 2-DG daily. Mice with EMT-6 breast tumors underwent FDG-microPET scans prior to and after 14 days of daily IP 2-DG which demonstrated similar uptake before and after treatment indicating that resistance to 2-DG uptake does not develop. Uptake of ^{64}Cu-PTSM was unchanged with 2-DG treatment. In tumor- bearing mice treated with 1mCi ^{64}Cu-PTSM and 40mg of 2-DG, tumor growth was inhibited approximately 80% compared to no treatment or treatment with ^{64}Cu-PTSM alone, and approximately 75% smaller than treatment with 2-DG alone. At a higher dose of ^{64}Cu-PTSM (2mCi) and 40mg 2-DG, survival at 20 days was 80% compared to 40% in mice treated with ^{64}Cu-PTSM alone and 20% in untreated mice.

Discussion: Our results indicate that tumor specific metabolic inhibitors such as 2-DG which cause cancer cell death can potentiate the effect of localized radiotherapy provided by ^{64}Cu-PTSM. We believe that combining tumor specific metabolic inhibitors with other cytotoxic agents will result in enhanced tumor killing with minimal effect on the host.

362 ANGIOZYME® treatment of stage IV metastatic breast cancer patients: assessment of serum markers of angiogenesis.

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Vascular endothelial growth factor (VEGF) mediates angiogenesis through two endothelial cell surface receptors, VEGFR-1 and VEGFR-2. ANGIOZYME, a chemically stabilized ribozyme, specifically targets VEGFR-1 mRNA. To evaluate the safety and efficacy of this anti-angiogenic ribozyme, we conducted an open-label Phase II trial of 45 previously treated Stage IV metastatic breast cancer patients. Twelve patients were treated for 12 weeks or more with daily subcutaneous doses of 100 mg/m^2 ANGIOZYME, however, no objective responses to ANGIOZYME monotherapy were seen. Sera from 31 patients were collected at baseline and after 6 weeks of treatment for analysis by ELISA of several endothelial cell related soluble (s) markers: sVEGFR-1, sVEGFR-2, sVCAM and sE-selectin. A significant reduction in the sVEGFR-1 level was noted in patients with detectable levels at baseline (174±40 pg/mL to 99±23 pg/mL, p=0.012). The sVCAM levels were significantly increased at 6 weeks compared to baseline (838±39 pg/mL to 663±34 pg/mL, p<0.001). In contrast, sVEGFR-2 and sE-selectin levels remained unchanged, as did plasma VEGF levels for the 26 patients evaluated. Because the ANGIOZYME target site is present in the pre-RNA encoding cell surface VEGFR-1 as well as the soluble form of the receptor measured here, these data suggest that ANGIOZYME has specific biological activity in patients with metastatic breast cancer. The decrease in sVEGFR-1 in the sera of ANGIOZYME-treated patients in this study did not correlate with clinical response. Further study of ANGIOZYME to assess its clinical utility in combination with other agents is warranted.

363 The role of p53 in 4-hydroxytamoxifen-resistant MCF-7 breast cancer cells *in vitro* and *in vivo*.

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Tamoxifen (TAM) is a selective estrogen receptor modulator that is currently the endocrine treatment of choice for all stages of breast cancer, however, TAM resistance eventually occurs. Many mechanisms for TAM resistance have been proposed and widely studied. We have developed new cell models and addressed a possible role of p53 in TAM resistance. **Methods:** MCF-7/E6 cells were generated by stably over-expressing human papilloma virus-16 E6 viral oncoprotein. After being exposed to 1 μM 4-hydroxytamoxifen (4-OHT) for > 12 months, MCF-7 and MCF-7/E6 cells became 4-OHT-resistant, resulting in MCF-7/4-OHT and MCF-7/E6/4-OHT cells. Both MCF-7/4-OHT or MCF-7/E6/4-OHT cells (10^7) were inoculated into athymic mice and treated orally with high dose TAM (1.5 mg daily) to determine tumor growth. **Results:** MCF-7/E6 cells grew at a faster rate than MCF-7 cells, similarly, MCF-7/E6/4-OHT cells grew at a faster rate than MCF-7/4-OHT cells *in vitro* as determined by DNA assays. The results from flow cytometry studies showed that MCF-7/E6 cells became resistant to 4-OHT in a shorter time period compared to MCF-7 cells. In MCF-7/4-OHT cells, the estrogen receptor alpha (ERα) was expressed at a similar level to that of MCF-7 cells. In addition, 17β-estradiol (E_2) induced ERE-luciferase activity and stimulated growth in these cells. Although the level of ERα in MCF-7/E6/4-OHT cells was also similar to that of MCF-7/E6 cells, both basal and E_2-induced ERE-luciferase activities were much lower in MCF-7/E6/4-OHT cells. Moreover, E_2 no longer stimulated cell growth in MCF-7/E6/4-OHT. Consistent with growth rates observed in cell culture, MCF-7/E6/4-OHT tumors grew faster than MCF-7/4-OHT tumors in athymic mice in the presence of TAM. **Conclusion:** Down-regulation of p53 by expression of human papilloma virus-16 E6 resulted in a faster growth rate, facilitated development of 4-OHT-resistance in MCF-7 cells, and enhanced the growth advantage of 4-OHT-resistant tumors in the presence of TAM.

Supported by the SPORE CA89018-02 and the Avon Foundation.

364 Mechanisms of tamoxifen resistance: long term tamoxifen exposure enhances the sensitivity of breast tumor xenografts to estradiol.

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Hormone dependent breast tumors initially regress upon tamoxifen (TAM) treatment but later regrow. We wished to test the possibility that tumors exposed to TAM long term may become hypersensitive to the estrogenic effects of TAM as well as estradiol (E2). Both in vitro and in vivo paradigms were utilized to study the effects of exposure to TAM over a several month period. The in vitro studies demonstrated an increased growth rate of wild type MCF-7 cells grown in the presence of 10(-7)M TAM but no remarkable change in level of E2 sensitivity as assessed by dose response testing. The in vivo paradigm utilized MCF-7 cells transplanted into oophorectomized nude mice. These xenografts were initially allowed to become established by the influence of small amounts of E2 delivered by silastic capsule. After tumors became measurable, E2 capsules were removed at 4 weeks. Animals were then divided into 2 groups. One received 25 mg implants of TAM and resulting tumors were called long term tamoxifen treated (LTTT). The other group was given cholesterol and xenografts were called control MCF-7 tumors (C-MCF-7). During this time period, the LTTT group regressed to a lesser extent than did C-MCF-7 tumors. After 4 months of TAM exposure, the LTTT appeared to begin to regrow as has been described by the Osborne and Jordan groups previously (Eur.J.Cancer, 1987,23,1189; Cancer Res.,1988,48,5183). At five months, TAM or vehicle implants were removed from all animals. The LTTT and C-MCF-7 subgroups were then each subdivided further into 3 groups which received either vehicle alone, E2 "clamped' at a plasma concentration at 1.25 pg/ml level or E2 at 20 pg/ml using methods previously validated by us (Shim et al., Endocrinology, 2000,141,396). Tumor growth curves were plotted in these animals during the next 7 weeks. Neither group responded to the very low dose of E2 (1.25 pg/ml). The LTTT cells did exhibit a marked enhancement of tumor growth upon exposure to 20 pg/ml of E2. In contrast, the C-MCF-7 cells did not respond to this dosage of E2 with increased growth rate. Thus, in the course of development of TAM resistance, the stage of increased sensitivity to the agonistic effect of TAM precedes the stage with adaptive higher sensitivity to E2. The latter is more evident in case of the *in vivo* model.

L.B. is a recipient of AstraZeneca UICC Translational Cancer Research Fellowship (TCRF 2001).

365 Effect of HER2 amplification status on the antiproliferative response of ER+ primary breast cancer to fulvestrant (Faslodex).

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Circumstantial evidence suggests that patients with ER+HER2+ breast cancer may respond poorly to tamoxifen and that this may be due to enhanced phosphorylation of ER as a result of kinases downstream of HER2. Fulvestrant (Faslodex) is a pure antioestrogen that down-regulates ER by destabilizing the protein. It might therefore not be subject to the same putative resistance mechanism. We analysed the HER2 amplification status by FISH (Vysis Pathvysion) in stored sections of pretreatment biopsies from a previously reported randomized, presurgical study of 14-21d fulvestrant (50 or 125 or 250mg im) or tamoxifen 20mg/d or placebo in postmenopausal women with primary breast cancer (Robertson et al, Cancer Res, 61, 6739-46, 2001). That study reported a dose-related decrease in levels of ER, PgR and the proliferation marker Ki67 in ER+ fulvestrant-treated patients. In the present study changes in were assessed in the ER+ non-placebo patients. A total of 113 biopsies were available; FISH analysis was not possible on 4 samples. Six tumours had >2-fold amplification (range 2.1-9.4). All were from fulvestrant-treated patients: 1 at 50mg, 1 at 125mg and 4 at 250mg (the dose approved for clinical use). In the fulvestrant-treated HER2+ and HER2- patients pretreatment median Ki67=26.7% (range 16.6-46.6%) and 11.9% (1.7-49.1%), respectively, p=0.002. Median fall in Ki67=11.3% and 41.4%, respectively, p=0.15. For the patients treated with 250mg fulvestrant median fall in Ki67=6.3% (n=4) and 56.0% (n=25), respectively. Thus there was no appreciable fall in proliferation in the HER2+ patients after this short exposure to the clinical dose of fulvestrant. Data on the clinical effects of fulvestrant in ER+ tumours with amplified HER2 should be sought.

366 Regulation of aromatase expression in preadipocytes by liver receptor homologue-1 (LRH-1) and small heterodimer partner (SHP).

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Estrogen biosynthesis from C_{19} steroids is catalyzed by the enzyme aromatase cytochrome P450. Aromatase is expressed in breast adipose tissue through the use of a distal, cytokine-responsive promoter (pI.4). In the presence of breast tumors, however, aromatase is over-expressed in response to tumor-derived factors that induce the proximal, cAMP-responsive promoter II (pII). In other tissues, transcription from promoter II requires the presence of Steroidogenic Factor-1 (SF-1, NR5A1). Adipose tissue, however, expresses little or no SF-1. We hypothesize that in adipose tissue, the related protein Liver Receptor Homologue-1 (LRH-1, NR5A2) substitutes for SF-1 in driving transcription from pII. Quantitative real time PCR and Western blot analyses revealed that LRH-1, but not SF-1, is expressed in human adipose tissue. LRH-1 (and aromatase) were co-expressed specifically in the preadipocyte fraction of adipose tissue, and differentiation of cultured human preadipocytes into mature adipocytes was associated with a rapid loss of LRH-1 and subsequently aromatase expression. Transient transfection studies in 3T3-L1 preadipocytes revealed that LRH-1 strongly stimulates expression of aromatase pII-luciferase reporter genes. This stimulatory effect required the presence of a nuclear receptor half-site within promoter II, to which LRH-1 was shown to bind in gel shift analysis. Finally, LRH-1-induced pII activity was dose-dependently and completely inhibited by co-transfection of the short heterodimer partner (SHP), an atypical nuclear receptor that lacks a DNA binding domain and represses activity of a range of other nuclear receptors. We conclude that LRH-1 is expressed at high level in human preadipocytes, and regulates aromatase transcription from pII. Alterations in the expression or activity of LRH-1 (or SHP) in breast adipose tissue by, for example, hormonal regulation or pharmaceutical intervention, could therefore have considerable effects on local estrogen production and breast cancer development.

367 EGF induces activation of estrogen response elements in an ER-independent but $p21^{Ras}$-dependent manner in MDA-MB-231 breast cancer cells.

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Background: There is evidence, that a crosstalk between estrogen receptor (ER) and receptor tyrosine kinase (RTK) signaling is present in tumor cells. ERs have been shown to be substrates of mitogen activated protein (MAP) kinases in MCF-7 breast cancer cells, whereas estrogens are able to activate RTK signal transduction components like MAP kinases. In this study, we intended to examine the importance of the expression of functional ERα for the growth-factor induced activation of estrogen response elements (EREs). Material and Methods: In order to examine the effect of epidermal growth factor (EGF) on activation of EREs, we performed reporter gene studies by means of transient transfection assays. MCF-7 and MDA-MB-231 breast cancer cells were transfected with the vector pERE-TA-SEAP coding for the secreted alkaline phosphatase (SEAP) gene under transcriptional control of estrogen response elements and with plasmid pCMVβ coding for the β-galactosidase enzyme for determination of transfection efficacy. ERE activation was determined by luminometric measurement of SEAP protein in the cell culture supernatant. Results: Our results demonstrate the H-ras-dependent but ERα-independent activation of EREs by EGF in an estrogen-unresponsive cell line, an effect which was not observed in the ERα/β-positive breast cancer cell line MCF-7. In MDA-MB-231 cells, the transcriptional activity of an ERE-containing promotor was enhanced dose dependently by all tested EGF concentrations. This effect could be blocked by co-treatment with the EGFR inhibitor AG1478, as well as by co-transfection with a vector coding for a dominant negative H-ras mutant, but not by co-treatment with the pure antiestrogen ICI 182,780. Furthermore, expression of constitutively active H-ras was shown to be sufficient to activate EREs in MDA-MB-231 cells. Discussion: In conclusion, our results demonstrate alternative utilization of ERE-mediated gene regulation in an estradiol-unresponsive breast cancer cell line in response to an EGF stimulus. This mechanism is dependent on EGFR and H-ras activity, but independent of the presence of functional ERα. Demonstrating an unexpected effect of EGFR inhibitors on activation of estrogen response elements, our data also suggest a possible dual role of EGFR inhibitors in the treatment of ERα-negative, EGFR-positive breast cancer.

368 Effects of anastrozole on lipid metabolism compared with tamoxifen and toremifene in ovariectomized rats.

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Introduction: Anastrozole (ANA), a new generation aromatase inhibitor, has been used to treat postmenopausal metastatic breast cancer, and several clinical trials of adjuvant treatment using this agent are ongoing. However, the effects of ANA on lipid metabolism are unknown. We previously reported that tamoxifen (TAM) decreases the activity of lipoprotein lipase (LPL), a key enzyme of triglyceride metabolism, in clinical and experimental studies (Hozumi et al, J Clin Endocrinol Metab 1998, Hozumi et al, Horm Res 2000). The aim of this study is to evaluate the effect of ANA on lipid metabolism, especially LPL activity, compared with selective estrogen receptor modulators (SERMs) in rats.

Methods: Ovariectomized female rats were divided into six groups: C, controls; TAM, tamoxifen treatment; TOR, toremifene treatment; ANA, anastrozole treatment; CAT, combined anastrozole/tamoxifen treatment; and AAT, anastrozle treatment after tamoxifen. The agents were orally administered for three weeks. Serum total cholesterol, triglycerides, and LPL activity in post-heparin plasma were measured at the end of the experiment.

Results: Serum cholesterol levels were significantly lower in the TAM, TOR and CAT groups than in controls ($P<0.001$). Serum triglyceride levels were significantly higher in the TAM group than in the other groups ($P<0.001$). LPL activity was significantly lower in TAM and AAT groups ($P<0.01$). There was no significant difference in any other parameters in group ANA.

Conclusion: Anastrozole does not affect lipid metabolism including LPL activity. There was little effect on lipid profiles during combination treatment or following treatment with tamoxifen. In clinical situation, therefore, anastrozole might be safe for patients with abnormal triglyceride profiles during SERMs treatment. A further clinical study is mandatory.

369 Hormonal activity of 4-hydroxyestradiol: characterization of specific 4-hydroxyestradiol binding.

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There are numerous recent indications of a possible physiological role for 4-hydroxyestradiol (4-OHE2) in hormone-responsive tissues, such as: a) the expression of a specific estrogen 4-hydroxylase in reproductive organs, such as breast and uterus, but not in liver, where most estrogen metabolism occurs; b) its essential role in blastocyst implantation; c) the 60-fold induction of lactoferrin expression in ERKO mouse uterus by 4-OHE2 compared to a doubling by E2 over control values.

To examine a hormonal activity of 4-OHE2, we have studied the binding of 3H-labeled 4-OHE2 (specific activity: 50 Ci/mmol) to mouse uterine cytosolic protein. These binding studies were conducted by incubating protein preparations with 2nM [3H]-4-OHE2, in the presence and absence of 200-fold excess (400nM) unlabeled 4-OHE2, E2 or 4-OHE2 + E2 (400nM each). In 3 week-old mouse uterus, total binding was 322.34 ± 24.16 fmol/mg protein. Binding in the presence of excess unlabeled 4-OHE2 dropped to 105.82 ± 29.0 fmol/mg protein, whereas 209.84 ± 32.19 fmol/mg protein bound while incubating in an excess of unlabeled E2. Binding in the presence of a combination of 4-OHE2 + E2 was no different from that in excess of 4-OHE2 alone. The difference between the two binding values (104.02 ± 30.6 fmol/mg protein) is considered to be selective binding of 4-OHE2 to a specific binding protein. 4-OHE2 binding displaceable by E2 approximately corresponds to levels of estrogen receptor measured previously. In younger mice (1 and 2 weeks of age) there was no detectable, specific binding by 4-OHE2, i.e. there was no difference between displacement by 4-OHE2 and displacement by E2. In older mice the specific 4-OHE2 binding slowly declined: 31.99 ± 3.99 fmol/mg protein at 8 weeks, 54.81 ± 6.31 fmol/mg protein at 12 weeks and 54.63 ± 5.2 fmol/mg protein at 9 months. Other organs tested (liver, kidney, heart and whole brain) showed no significant selective binding of 4-OHE2.

These results lead us to conclude that 4-OHE2 binds to a specific binding protein, in addition to its binding to estrogen receptor(s) (ERα and ERβ). The physiological role of this binding remains to be elucidated.

Supported by NIH, NCI 74971.

370 Bone mineral density and hormone receptor status in patients with newly diagnosed breast cancer, a retrospective assessment.

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Background: High endogenous estrogen (E) levels have been noted in obese women and increased E exposure correlates with both increased bone mineral density (BMD) and estrogen receptor (ER) positive breast cancers (BCA). Selective estrogen receptor modulator use is associated with a decrease in the risk of both osteoporosis and ER or progesterone receptor (PR) positive BCA. Hence, BMD may serve as a surrogate marker for assessing a woman's lifetime E exposure and therefore her risk of developing BCA. We sought to investigate this association.

Materials and Methods: Patients (pts) diagnosed with operable, primary BCA after 1/2/01 and who underwent assessment of BMD by Dual Energy absorptiometry (DXA) at MSKCC between 1/07/02 and 5/15/02 were identified. All charts were reviewed for tumor stage, hormonal receptor status and DXA results. Normal BMD is less then or equal to 1 standard deviation (SD), osteopenia is between 1 and 2.5 SD and osteoporosis 2.5 SD or less below the mean for the young adult population (T score).

Results: 53 BCA pts with BMD assessment at MSKCC were identified. Median age 60 years (range 36-82, mean 59). Tumor stages are T1-T4; N0-N1; M0. 37 pts (70%) received adjuvant chemotherapy at MSKCC. 21 pts had normal BMD DXA results and 32 pts had a DXA T score of less than -1.0 at the hip, femoral neck or spine. 14 pts (26%) had tumors that were ER and PR negative; the remaining 39 pts (74%) had ER and or PR positive tumors. In the ER/PR negative subset, 8 pts (57%) had DXA scans demonstrating osteopenia or osteoporosis. In the ER and/or PR positive subset, 24 pts (62%) had DXA scans demonstrating osteopenia or osteoporosis.

Discussion: In our retrospective chart review, DXA results from within 1 year of diagnosis of primary BCA identified osteopenia or osteoporosis in approximately 60% of pts regardless of tumor ER or PR status. Although DXA results may have been influenced by BCA therapy, our data do not support the hypothesis that BMD and BCA risk or receptor status are linked.

371 Effect of tibolone and its two hydroxy metabolites on estrone sulfatase activity in human breast cancer tissue.

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Background: There is abundant evidence that breast cancer tissue contains the enzymes necessary for the formation of estradiol (E2) from circulating precursors, including sulfatase, aromatase, and 17β-hydroxysteroid dehydrogenase. Two main pathways for estrogen formation in breast tumors have been characterized: the "aromatase", which converts circulating androgens into estrogens and the "sulfatase", which converts estrone sulfate (E1S) into estrone (E1). Previous studies in this laboratory demonstrated that various progestins (e.g. nomegestrol acetate, medrogestone, promegestone) have an antisulfatase activity in various hormone-dependent breast cancer cells. It was also demonstrated that tibolone and itsmetabolites (3a-OH-tibolone, 3β-OH-tibolone and the D4 isomer) are potent antisulfatase agents in these cell models. In the present study, we explore the effect of tibolone on the sulfatase activity using the total breast cancer tissues.

Materials and Methods: Slices of breast tumoral tissues (25-40 mg) were incubated in buffer (20 mM Tris-HCl, pH 7.2) with physiological concentrations of [^{3}H]-estrone sulfate ($5x10^{-9}$M) alone or in the presence of tibolone or its metabolites: 3β-OH-tibolone and 3a-OH-tibolone ($5x10^{-5}$ to $5x10^{-7}$) during 4h at 37°C. E1S, E1 and E2 were characterized by TLC and quantified using the corresponding standard.

Results: Tibolone and its metabolites: 3β-OH-tibolone and 3a-OH-tibolone inhibit the conversion of E1S to E1 by 44%, 74%, and 71% respectively at the concentration of $5x10^{-5}$ M. At the low concentration of $5x10^{-7}$M the sulfatase inhibition is 30% and 37% respectively for 3β-OH-tibolone and 3a-OH-tibolone but only 19% for tibolone. In these experiments it was observed that E1 does not convert to E2.

Conclusion: It is concluded that tibolone, as well as 3β-OH-tibolone and 3a-OH-tibolone can also inhibit the sulfatase using the total breast tissues.

372 Localization of enzymes needed for the biosynthesis of catecholestrogens and enzymes that can prevent their oxidation to their potentially mutagenic quinone metabolites in normal and neoplastic human breast parenchyma.

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Chronic oxidative stress in breast parenchyma is implicated in the high breast cancer incidence in the USA. A mechanism by which catecholestrogens (CEs) may contribute to oxidative cellular and DNA damage via their quinone metabolites (QEs) has been well characterized. Its relevence to carcinogenesis is indicated by several lines of evidence, including formation of depurinating DNA adducts by QE metabolites of 4-OH-CEs and mutations in MCF10F cells exposed to estradiol (E2) in vitro. Identification of 2- and 4-OH-CEs in human breast tissue points to the relevance of the CE/QE pathway of estrogen metabolism to breast carcinogenesis. Objective: To test the hypothesis that enzymes needed to generate CEs and to prevent their oxidation to QEs are co-expressed in human mammary epithelium. Methods: Immunolocalization in normal and neoplastic human breast parenchyma, using antibodies of demonstrated specificity, of four enzymes relevant to CE homeostasis: a) P4501B1 and P4503A4, enzymes that can catalyze the hydroxylation of estrone (E1) and E2 at C4 and C2, respectively, and; b) catechol-O-methyltransferase (COMT) that can block oxidation of 2- and 4-OH-CEs to their respective QEs, and the glucuronosyltransferase UGT2B7 that can block oxidation of 4-OH-E1. Results: Immunostaining (IST) for all the enzymes was present in mammary epithelial cells throughout the normal mammary epithelium, but was much reduced or absent in highly invasive cancers. In many in situ lesions, however, IST was more intense than normal. Conclusions: Enzyme needed to generate CEs are co-expressed in mammary epithelial cells, as are ones that can block their oxidation to QEs. Diversion of CEs into the rogue CE/QE pathway due to an imbalance between the rate of generation and inactivation of CEs could result in genomic damage. Increased CE formation may follow induction of P450s e.g., of P4501B1 by dioxins, and protection against CEs oxidation could become compromised by competition for COMT or UGT2B7 by other substrates. The striking difference between in situ and invasive cancers suggests a dynamic involvement of these enzymes relevant to CE homeostasis not only in the genesis but in the progression of breast cancer.

373 Preclinical studies on enhancement of cytotoxic effect of anticancer drugs in combination with antisense Bcl-2 in breast cancer.

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[Backgrounds and Objectives]: Down-regulation of Bcl-2, an antiapoptotic protein by antisense Bcl-2 oligonucleotides (ODNs) enhances therapeutic efficacy of anticancer drugs in various cancers including prostate, lung, leukemia, melanoma, which are under phase II/III clinical trials. In the present study, we assessed therapeutic efficacy of antisense Bcl-2 in breast cancer cells transplanted into nude mice in combination with anticancer drugs. [Materials and Methods]: Using three breast cancer cell lines (BT-474, MDA-MB-231, MDA-MB-453), which are differentially expressed in Bcl-2 protein, we examined preclinical studies on enhancement of drug-sensitivity by pretreatment of antisense Bcl-2. The used antisense was phosphorothioated ODNs of 18 mers, and it was preadministered intraperitonealy with dose of 5mg/kg for consecutive 6 days. Anticancer drugs including MMC, TXL, TXT, 5-FU, ADM were administered at day 6 with LD50 /3 following the antisense in 14 days in one cycle, and repeated twice. Antitumor effect was evaluated by NCI protocol. Expression of apoptosis-related protein was assessed by Western blotting and immunohistochemical staining. Apoptotic cell death was assessed by TUNEL method. [Results]: The specific down-regulation of Bcl-2 protein treated with the antisense Bcl-2 was confirmed in 60%~70% as compared to that of control ODNs. The cytotoxic effect to the all drugs tested was significantly enhanced by pretreatment of the antisense Bcl-2 with greater extent to taxanes in the breast tumors, although monotreatment of the antisense somewhat suppressed the tumor growth. Antisense Bcl-2-enhanced antitumor effect was observed in accordance with the level of Bcl-2 protein. The enhanced antitumor effect was associated with increase of apoptotic cell death, which was induced in part by inhibition of Bcl-2 phosphorylation by taxanes. [Conclusion]: Overexpression of Bcl-2-mediated drug-resistance can be overcome by pretreatment of the antisense Bcl-2, which causes down-regulation of Bcl-2 and inhibition of Bcl-2 phosphorylation leading to apoptosis.

374 Dual genetic reporter system for early diagnosis and monitoring of breast cancer.

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Introduction.We have developed a genetic dualistic reporter system for early diagnosis and monitoring of human breast cancer. Our system uses an Adenoviral (Ad) vector to transfer the genetic reporters to the breast cancer. Infection of the cancer cells leads to expression of one reporter that can be detected in blood, namely, secreted human placental alkaline phosphatase (SEAP). A second reporter, enhanced green fluorescent protein (GFP), is also delivered by the Ad, leading to expression at the site of breast cancer. Methods. The SEAP gene under control of the CMV promoter element was linked to the GFP gene with an IRES element. An Ad vector encoding the SEAP and GFP (Ad-SEAP-GFP) was produced. Ad-SEAP-GFP-infected MD468 were monitored over time for fluorescence and SEAP was also measured in the growth media supernatants. MD468 cells were implanted in nude mice (n=5/group), either (SC, group 1) or (IP, group 2). On 15th d the Ad-SEAP-GFP (5x109 particles) was directly injected group 1, and IP for group 2. Additional mice without IP tumors (Group 3) were injected IP with the same dose of Ad-SEAP-GFP. Mice were imaged with a stereomicroscope after 24 h, and SEAP was assayed from blood samples. Group 2-3 mice were killed at 24 h, Group 1 mice were followed until 4 days. Results. Increasing GFP fluorescence was observed in MD468 cells infected with Ad-SEAP-GFP, while SEAP levels in growth media increased over the 12 day monitoring period (max=930 mg/ml). Expression of GFP in both SC and IP tumors was observed by 24 h in the live mice and SEAP blood levels were more than 10-fold greater than that of control group 3. At 24 h, the peritoneal washings of group 2 were nearly 5-fold higher than the levels in group 3 mice. GFP fluorescence and SEAP levels continued to increase in group 1 mice until termination. Conclusions. These experiments establish the feasibility of combining two genetic reporters for the early diagnosis and monitoring of human breast cancer. The novelty of the dualistic system is the linkage of blood-based reporter screening as a selection criteria for subsequent light-based imaging procedures. Future efforts will be directed to targeting the Ad vector to more specifically infect only breast cancer cells, with expression of the reporters controlled by breast cancer specific promoter elements.

375 Antitumor efficacy of anaerobic bacteria as delivery system for breast cancer gene therapy.

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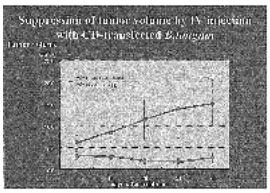
Background: A fundamental obstacle in breast cancer gene therapy is the specific targeting of therapy directly to a tumor, and no systemic delivery system yet exists. A strain of domestic bacteria, *Bifidobacterium longum*, which is nonpathogenic and anaerobic, selectively localized to and proliferated in solid tumors after systemic application. We proposed a novel approach to cancer gene therapy in which anaerobic bacteria of the genus *Bifidobacterium longum* (*B. longum*) are used to achieve tumor specific gene delivery and enzyme-prodrug therapy.

Materials and Methods: Female Sprague-Dawley rats ,6 weeks old were used in this study. As autochthonous tumor, the rats were administered 10 mg of 7,12-dimethylbenz[a]anthracene(DMBA) by intragastric gavage weekly for two weeks. At 23 weeks after the first dose of DMBA ,89% rats developed mammary tumors. We chose to use the combination of cytosine deaminase (CD) and 5-fluorocytosine (5FC) in initial studies of the feasibility of this strategy. We constructed pBLES100-S-eCD that includes HU gene promoter and cytosine deaminase gene in shuttle vector pBLES100. Rats bearing chemically induced mammary tumors received a 4-day regimen of CD-transfected *B.longum* in tail vein. The rats were administered 500 mg of 5FC per kilogram per day by intragastric gavage. Tumor size in injected group was compared with that in non-injected group.

Results: Tumor size in the control group (n=5) tended to be increased, while tumor sizes in the injected group (n=11) tend to be stable. 76 days after administration, tumor size in the control group was significantly larger than that of the injected group.

Discussion: *B.longum* is specifically grown in hypoxic tumors, making it effective as novel vector for gene delivery. Transfected *B.longum* produced cytosine deaminase in the rat mammary tumor. It was confirmed to be effective for Prodrug-Enzyme therapy of systemic administration of breast cancer patients.



376 DNA nanoparticles for breast cancer gene therapy.

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DNA transport through the cell membrane is an essential requirement for gene therapy that utilizes oligonucleotides and plasmid DNAs. However, DNA uptake by cells is an inefficient process, prevented by several barriers. Viral vectors provide an efficient means for delivering DNA to cellular targets; however, the immune response elicited by viral proteins is a serious limitation. Therefore, there is an increased emphasis on developing nonviral DNA delivery vehicles for gene therapy. It has been recognized that the first step in the interaction of nonviral vectors with DNA is the collapse of DNA to nanoparticles. The mechanism of DNA nanoparticle formation is not well established, although electrostatic interactions between the DNA and delivery vehicles, which are generally polycationic molecules, appear to be the primary process of DNA compaction. In an ongoing effort to develop novel DNA delivery vehicles, we studied the ability of a series of pentamine and hexamine analogs of the natural polyamine, spermine, to provoke the condensation of a luciferase-tagged plasmid DNA, pGL3 control vector, using laser light scattering and atomic force microscopy techniques. Using total intensity light scattering, we found that the plasmid DNA underwent a facile transition to the compacted state at 1-5 μM concentrations of the pentamine and hexamines in a buffer containing 10 mM Na cacodylate (pH 7.4). There was an increase in the concentration of polyamines required to collapse DNA as the concentration of Na+ increased. Dynamic laser light scattering studies showed that the size of the collapsed DNA was in the range of 50-100 nm. Atomic force microscopy demonstrated the morphology of the DNA particles as toroids and spheroids. Similar types of DNA nanoparticles were formed with other nonviral delivery vehicles, including polyethylenimine and polylysine. We found that these agents could transport the plasmid DNA and oligonucleotides in breast cancer cells. Taken together, DNA nanoparticle formation is an important aspect of developing novel DNA delivery vehicles for breast cancer gene therapy.

377 Inhibition of breast tumor progression by systemic delivery of maspin in a syngeneic tumor model.

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Background Maspin has been shown to possess tumor-suppressing activity against breast tumor growth and metastasis. To test maspin's therapeutic value in breast cancer, we established a syngeneic breast tumor metastasis model. This model involved the implantation of mammary tumor cells orthotopically to mammary gland and tumors were allowed to grow within the gland and become metastatic to other organ.

Materials and methods The mammary tumor cells were initially isolated from MMTV-polyoma virus middle T transgenic mice and were selected in vitro for high invasiveness. PyV mT-high cells were grown to 70-85% confluence before being harvested for cell counting. Cells were implanted to the #4 mammary gland of syngeneic mice tumor growth rate was monitored every two days by caliper measurement. For gene delivery, the human maspin cDNA was constructed in a expression vector. The pEF-maspin and its pEF vector control were mixed with liposome for systemic treatment. The delivery of maspin transgene to mammary tumors was verified by RT-PCR assay.

Result We demonstrated that the mammary tumor cells (PyV mT-high) were highly invasive and metastatic. Overall, 100% of tumor transplanted mice developed lung metastasis. Using non-viral liposome as a carrier, we delivered maspin gene to mice bearing mammary tumors. Our data showed that both primary tumor growth and metastasis were significantly inhibited in this syngeneic metastasis model. Such inhibition is mediated by maspin transgene through increased apoptosis in maspin treated tumors. Thus, maspin can be used in gene therapy against breast tumor growth and metastasis.

Discussion Cancer gene therapy requires both a good animal model and an effective drug delivery system. There are several systems available for gene delivery. Among them, a nonviral and nontoxic delivery system seems to have certain advantage and thus is used in this study. There are few animal models available for tumor metastasis study. Several transgenic tumor models were established previously. However, there are some limitations associated with these models. One is that the time for tumor development in each animal varies greatly in individual animal, depending on the activation of its transgene. This will affect our evaluation of the treatment efficacy since all reagents are preferred to be delivered to the group of animal at the same time. To circumvent such problems, we established a syngeneic breast tumor model and to carry out gene delivery study in this new model. Our data demonstrated the utility of this animal model and the effectiveness of maspin:liposome treatment against breast tumor progression.

378 Adeno-associated viral (AAV) vector serotypes transduce a broad host range of human breast cancer cell lines.

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Adeno-associated viral (AAV) vectors have become increasingly popular delivery vehicles for gene transfer studies. The reasons include their lack of pathogenecity and immunogenecity in animal models and humans. Relatively little information is available concerning the transduction efficiency of AAVs in human models of cancer. Here, we have compared the six identified serotypes of AAV vectors for transduction efficiency in five breast cancer cell lines to assess the host range of these vectors. The cell lines chosen varied genotypically and phenotypically. AAV vectors encoding GFP, Alkaline phosphatase or lacZ reporter genes were used to transduce glioma and breast cancer cell lines grown as monolayers. The transduction efficiency was assessed by histochemistry, light microscopy and flow-cytometry. Following transduction for three days, all cell lines were permissive to the AAV serotypes albeit to varying degrees. This data suggested that all AAV vector serotypes have a broad host range for breast cancer cells and that the receptors for these various AAV serotypes are widely expressed on breast cancer cells. We next generated an in vivo model to see if these AAV vectors were effective in vivo. In xenografts (prepared from glioblastoma spheroids implanted into cerebral regions), we injected serotype vectors and following two months, we observed transduction in both peripheral and central regions of the in vivo tumor. We conclude that AAVs represents an effective vector system that has a broad host range and are effective delivery systems capable of pentrating solid human tumor tissue in vivo. These results suggest a positive role for AAV technology in oncological therapeutic gene delivery.

379 Gene expression profiling of breast carcinomas.

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Introduction: A more detailed molecular information about breast carcinomas is needed to extend our understanding of the development and progression of the disease, and to potentially improve individual treatment. A novel classification of breast tumors through gene expression profiles may be helpful in reaching this goal. The purpose of this study is to examine gene expression profiles of tumors from a series of breast cancer patients, and correlate to clinical and histopathological parameters.

Material/method: Total RNA was isolated from frozen tumor tissues collected from a series of breast cancer patients. The tot RNA from 64 tumor samples and 11 normal breast tissue samples was amplified, labeled with Cy5 and co-hybridized with amplified Universal Human Reference RNA labeled with Cy3 to cDNA microarrays containing 44000 clones. The data analysis was performed using a hierarchical clustering algorithm.

Result: Preliminary analysis show that the samples cluster into at least three distinct groups based on their gene expression pattern; a normal-like, a basal-like and a luminal/ER+ -like group as previously found (Perou, C.M. et al., Nature, Aug.17, 2000; Sørlie, T., PNAS, Sept. 11, 2001.) The normal-like subgroup includes samples from normal breast tissue. Genes like keratin 5/17 show increased expression both in the normal-like and basal-like subgroup. Samples in the basal-like and luminal-like subgroup have increased expression level of genes involved in mitotic spindle checkpoint (e.g. BUB1 and MAD2). In contrast to the normal- and basal-like subgroup that show low expression of ER (estrogen receptor), the luminal/ER+ -like subgroup has increased expression of ER and ER regulated genes. Biochemical analysis confirms that they are ER+.

Future plans: Relation of the expression patterns to various clinical and histopathological data like survival, TNM, type and grade will be performed. An important aim of this study is to investigate whether the invasive lobular and ductal breast carcinomas, which differ histologically but are treated similarly, can be distinguished by gene expression profiling. Furthermore, the genomic instability found in breast carcinomas will be investigated, both by looking at the gene expression pattern of genes involved in the mitotic spindle checkpoint and mutations in genes believed to regulate this process.

380 Expression analysis of lobular and ductal carcinomas of the breast.

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Background: Ductal and lobular carcinomas are classified on the basis of their distinct histologies. Chromosomal CGH analysis of these tumors has shown distinct patterns of DNA changes. High resolution array based genomic techniques allows the grouping of related tumors based on expression and DNA copy number profiles, which can be correlated to clinical parameters. These types of analyses can identify genes driving formation of specific tumor types, identify potential therapeutic targets, and aid in prognosis.

Materials and Methods: DNA and total RNA were isolated from frozen breast tumors by standard methods. RNA was labeled with Cy3 dye and hybridized against a Cy5 labeled reference pool of 11 cell lines on 10K cDNA microarrays. Data was analyzed using GenePix, excel, and Cluster/Treeview software. Tumor DNA was labeled with Cy3 dye and hybridized against Cy5 labeled normal DNA on 2.4K BAC microarray slides. Data was analyzed using UCSF Cancer Center SPOT/SPROC software.

Results: Clustering of tumor samples based on their expression profiles does not distinguish lobular from ductal breast carcinomas. This clustering does reveal gene families that cluster together, such as proliferation specific, ERBB2-related genes, and estrogen receptor related genes. Tumor clusters based on expression profiles may be clinically significant, since the different clusters appear to have different disease free survival rates. Preliminary comparisons of gene expression data to DNA copy number data reveals that some large-scale amplifications correlate well with concomitant increases in gene expression.

Discussion: Expression analysis of lobular and ductal carcinomas fails to distinguish the two types of tumors, indicating that supervised clustering may be necessary to classify the two tumor types and identify tumor specific genes. Clustering of expression data appears to identify a subset of tumors with a worse prognosis. Comparison of array CGH profiles and expression profiles demonstrates that some large-scale amplifications correlate with increases in gene expression. These data suggest that the overall gene expression in lobular and ductal carcinomas is not significantly different and that subtle changes in expression may be responsible for the different histologies.

381 Molecularcytogenetic investigations of synchronous bilateral breast cancer.

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Background: Bilaterality in breast cancer points in context with an early onset of disease towards an inheritance of the disease. Nevertheless bilateral breast cancer also occurs in higher age, either in a synchronous or metachronous manner.

Material and Methods: 16 patients with synchronous bilateral breast cancers (both tumours diagnosed within 6 months) were included in the study. The average age was 68.5 years (range 41-89 years, median 68 years). DNA was isolated from paraffin-embedded material of 39 invasive or preinvasive breast cancers. These were investigated by means of Comparative Genomic Hybridization (CGH) and PCR-based Multiplex-Microsatellite analysis using a panel of 11 polymorphic markers. The results were analyzed conventionally.

Results: On average 4.1 alterations (range 0-14) per case were revealed by CGH. Chromosomal gains were most commonly seen on 1q (52%), 8q (26%), 17q (12%) and 20q (7%), whereas the majority of chromosomal losses could be detected at 2q (17%), 6q (29%), 8p (17%), 11q (26%), 16q (52%) and 17p (19%). Frequencies of "Loss of heterozygosity" (LOH) varied from 18% (egfr) to 91% (D16S400) with a median frequency of 50%. In detail: egfr 18%, caveolin 1+2 50%, D8S258 54%, NEFL 75%, PTEN 20%, Rb1 36%, D16S400 91%, D16S402 75%, D16S422 75%, p53 32%, BRCA1 21%.

Discussion: The average number of genetic alterations per case in these cases was lower than reported before in unilateral, sporadic breast cancer. In addition, the rate of 16q-losses as another indicator of tumour grade and the low number of tumours with high-level-gains indicating amplifications also points to a better "genetic differentiation" in bilateral breast cancer cases with a late onset of disease. The frequency of LOH in the markers investigated was similar as described in the literature. An involvement of these genes in the carcinogenesis of synchronous bilateral breast cancer is unlikely. A distinct, characteristic genetic alteration associated with bilateral breast disease could not be found, nevertheless the possibility of a specific susceptibility in the investigated cases remains open.

382 Genomic relationship of lobular carcinoma in situ and synchronous invasive lobular cancer.

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Background: Lobular carcinoma in situ (LCIS) of the breast has been thought to be a marker for increased cancer risk in both the ipsilateral and contralateral breast. However, the frequent association of LCIS with invasive lobular cancers (ILC) suggests a possible precursor-product relationship between LCIS and ILC. We undertook this study to determine the genomic relationship between LCIS and ILC using array-based comparative genomic hybridization (CGH).

Methods: 9 cases from the UCSF tumor bank containing synchronous LCIS and ILC were analyzed and manually microdissected. Genomic profiling by array CGH was performed by DNA random prime amplification and random prime labeling with cy3 conjugated d-UTP. Samples were hybridized to cy5 labeled reference DNA on BAC arrays. Concordance between matched pairs of LCIS and ILC was determined.

Results: The average number of genomic alterations was greater for ILC than for LCIS (10.7 vs. 5.9). 100% of LCIS and ILC specimens showed 1q gain and 16q loss. 3/9 matched pairs showed sufficient similarities in their patterns of genetic alterations to suggest a clonal relationship. 5/9 matched pairs had significantly different patterns of alterations.

Genomic Alterations in Synchronous LCIS and ILC

Case	Changes in Common	Changes in LCIS Only	Changes in ILC Only	% Concordance
LC02	1q+, 16q-, 17p-		1p-, 3+, 5p-, 5q+, 6q-, 7+, 9+, 10+, 11+, 15p-, 16p+, 17q-, 18-, 20+, 21+, 22-	27
LC03	1q+, 16q-, 5p+, 5q-, 8p-, 8q+, 11q-, 22-*		17p-	94
LC04	1q+, 16q-,1p-, 3q-, 11q-, 11q+, 13-	1q+, 16q-,1p-,3q-, 11q-, 11q+,13-	1p+, 8p-, 8q+, 16p+	74
LC06	1q+, 16q-		11q+, 11q-, 16p+, Xp+	50
LC07	1q+, 16q-, 8p-, 17p-	8q+, 13-, 17q+, Xp+	6-, 9+	57
LC12	1q+, 16q-, 8p-	12p-	6p-, 7+, 17p-	67
LC19	1q+, 16q-		1p-, 7p-, 8q-, 9-, 11-, 12p-, 16p+, 18-, 22-	31
LC39	1q+, 16q-, 11q+, 11q-, 17p-, 22q-		17q+	92
LC43	1q+, 16q-,1p-		3p-, 5q+, 6p-, 7p+, 7q+/-/+, 8p-, 11q+/-, 12+, 13-, 16p+, 16q-, 17p-, 18+, 19p-, 19q+, 20+, 21q-, 22-	23

Conclusion: High resolution array-based CGH confirms that LCIS is a neoplastic lesion. Some genomic alterations are seen in both LCIS and ILC, suggesting the same mechanism for early neoplastic progression in both lesions. Some, but not all matched pairs of LCIS and ILC show a high level of genomic concordance.

383 Estrogen-receptor independent induction of loss of heterozygosity in human breast epithelial cells by estrogen and metabolites.

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Background: We have demonstrated that 17 B-estradiol (E2), its metabolite 4-hydroxy-estradiol (4-OH-E2), and to a lesser degree 2-hydroxy E2 (2-OH-E2), induce phenotypic changes indicative of neoplastic transformation in MCF-10F, an estrogen receptor (ER) negative human breast epithelial cell (HBEC) line. MCF-10F cells treated with E2, 4-OH-E2, or 2-OH-E2 exhibited loss of ductulogenesis in collagen, increased invasiveness, and colony formation in agar-methocel, phenotypic changes that were not abrogated by treatment with the antiestrogens tamoxifen or ICI 182,780. This phenomenon, combined with the responsiveness to estrogen treatment by this ER negative cell line, was indicative that the effects observed were mediated through an ER independent mechanism. To further clarify their mechanism of action, we analyzed genomic DNA of treated cells for determining whether the induction of transformation phenotypes was associated with the induction of genomic changes. Material and Methods: MCF-10F cells were treated with 70 nM E2, 4-OH-E2, or 2-OH-E2 for four 24 hr. periods in two weeks, followed by 7-8 passages in hormone-free medium before harvesting and DNA extraction. Benz(a)pyrene (BP)- and progesterone (P)-treated cells served as cell transformation positive and negative controls, respectively. Genomic DNA was analyzed for the detection of micro-satellite DNA polymorphism using 64 markers covering chromosomes (ch) 3, 11, 13 and 17. Results: Loss of heterozygosity (LOH) in ch13q12.2-12.3 (D13S893) and in ch17q21.1 (D17S800) was detected in E2-, 2-OH-E2-, 4-OH-E2-, and BP-treated cells. E2- and 4-OH-E2-, like BP-treated cells, also exhibited LOH in ch17q21.1-21.2 (D17S806). Discussion: Our results indicate that E2 and its metabolites induce in ER negative HBEC the same genomic changes that are induced by a carcinogen, in addition to phenotypic changes of cell transformation. The loci affected are the same reported in primary breast cancer.

384 Polysomy of chromosome 17 in breast cancer with c-erbB2 overexpression: a study of 179 cases using fluorescence in situ hybridization (FISH).

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Introduction. The HER2/neu protooncogene located at 17q12-21 is overexpressed in 25-30% breast cancers and it is known that confers important prognosis and predictive value. The overexpression of this growth factor receptor is mostly caused by HER2/neu gene amplification in >90% of breast cancers. There are different methods to evaluate c-erbB2 overexpression/ amplification, but immunohistochemistry (IHC) and FISH are the most used. IHC and FISH have shown discordances in cases with increased chromosome 17 copy number per cell. Chromosomal aneusomy, either monosomy or polysomy, is frequently observed in breast carcinoma. However, it is unclear whether polysomy 17 may play a role in HER2/neu gene dosage and c-erbB2 overexpression.

Aims. We analyzed polysomy of chromosome 17 to know the contribution of this aberration to c-erbB2 overexpression, especially in cases with low levels of overexpression (IHC 2+).

Patients and Methods. We present a series of 179 patients studied prospectively in whom we performed IHC with Herceptest (DAKO) and FISH with PathVysion probe (Vysis) essentially in cases with score 2+ by IHC (113 patients).

Results. Among 179 patients we observed 12% (21/179) of polysomy 17. We found that 15% (17/113) of cases with IHC 2+showed polysomy 17. Cases with chromosome 17 polysomy have increased HER2 gene copies, but true gene amplification is presented only in 4% (7/179) of patients. We did not find different polysomy 17 distribution in cases with or without amplification (Table 1).

Conclusions.

-FISH analysis permits the confirmation of HER2/neu gene amplification.

-Polysomy 17 was more frequently found in cases scored 2+ by IHC.

-It is necessary to study in depth the clinical significance of polysomy 17.

Table 1.

IHC Score	Polysomy 17/ FISH Normal	Polysomy 17/ FISH Amplified	% Polysomy
0	0/5	—	0/5 (0%)
1+	0/28	—	0/28 (0%)
2+	13/84	4/29	17/113 (15%)
3+	1/2	3/31	4/33 (12%)
TOTAL	14/119 (12%)	7/60 (12%)	21/179 (12%)

Polysomy 17 according to immunohistochemical scoring and FISH

385 Multiple splice-variants of Chk2.

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Background: After double-stranded DNA-damage ATM activates the checkpoint kinase Chk2 by phosphorylation, followed by Chk2 oligomerisation, auto-phosphorylation and disassembly of oligomers. The fully activated Chk2 stabilizes p53 upon activation leading to p53-dependent growth arrest or apoptosis. Patients defective in Chk2 share the same phenotype as p53-defective patients, as no p53-activation occur after genotoxic stress. Several mutations of Chk2 have been described, but so far no reports on alternative splicing, and its implication in Chk2 activity, have been published.

Materials and Methods: Using purified RNA from breast cancer tissue samples and breast cancer cell lines we synthesized cDNA and analyzed Chk2 RNA-status by nested PCR. The PCR-products were cloned into the pCR TOPO 2.1 vector (Invitrogen) and sequenced. Selected splice-variants were verified by RNAse Protection Assay (RPA). Activity was measured as incorporation of radioactive phosphate in p53 and Chk2.

Results: After electrophoresis of the nested Chk2-PCR, several bands were observed in addition to wildtype Chk2. Sequence analysis revealed these to be alternatively spliced variants of Chk2. We observed about 70 new splice-variants, of which 50% were found in at least 2 patients. In addition, the different splicing events combined to form a series of multiple spliced variants bringing the total number of variants to ~100. 47 of the variants had new splice sites, of which 40 did not have the GT-AG intron-sequence. Of these variants, 80% of the occurrences had the intron sequence [A(…)G] or [C(…)T]. Selected variants were transfected into cell lines and tested for activity and subcellular localization. One variant, del2-3 was found to have significant activity (60% of wildtype levels).

Discussion: After Chk2-activation by ATM all domains of Chk2 is involved in a series of auto-phosphorylation events necessary for full Chk2 activity. There are several possibilities regarding the function of the splice-variants. It is important to note that normal activation of the Chk2 kinase activity is only found in the one tested variant, del2-3, which retain all functional domains of wildtype Chk2. Though all novel splice variants may not be biologically active, a splice-variant might dimerize with wildtype Chk2 during the activation steps. These dimers may not disassemble, making the splice-variant an inhibitor of wildtype Chk2 in the patient. The splice-variants may also inhibit Chk2-activity by for example preferentially binding ATM in its substrate-site. Multiple splicing is a feature of many proteins, and in the case of Chk2 it might be an alternative to mutations in disabling the p53 pathway.

401 Serum HER-2/neu levels correlate with time to progression in therapy of metastatic breast cancer.

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Background: Several previous studies suggested a correlation between high serum HER-2/neu (S-HER2) levels and poor prognosis in patients with metastatic breast cancer. The purpose of this study was to investigate a possible correlation between S-HER2 levels and responsiveness to antitumor therapy in these patients.

Patients and Methods: From 313 patients a total of 2964 serum samples (median: 8 per patient) drawn during the whole course of disease, was analysed retrospectively by Bayer Immuno 1™ assay for S-HER2 concentration. 15 ng/ml was chosen as cut-off level for S-HER2. Patients had received up to 6 regimens of chemotherapy and up to 4 endocrine therapy regimens and were assigned to three groups according to S-HER2 levels: Group 1 with continuously elevated (>15 ng/ml) S-HER2 values, group 2 with continuously normal (≤15 ng/ml) S-HER2 values during the entire course of disease, and group 3 with both normal and elevated S-HER2 values. Response to therapy was evaluated according to WHO criteria. Time to progression (TTP) was defined as time from start of therapy to objective disease progression or death.The correlation between S-HER2 levels with response and TTP was evaluated using the Mann-Whitney test and Chi-Quadrat test respectively.

Results: Tumor response did not differ significantly within the three groups.The median overall TTP for the sequential therapies in patients of group 1 (continuously elevated S-HER2 levels) was less than one month, in patients of group 2 (continuously normal S-HER2 levels) 2.8 months and 4.0 months in patients of group 3 (both normal and elevated S-HER2 values).The difference of median TTP in group 1 compared to group 3 as well as TTP in group 1 compared to group 2 was statistical significant. (p=0.001, p=0.05 resp.). There was no significant difference between group 2 and group 3.

Conclusion:Patients with elevated S-HER2 levels during the whole course of disease have a significant worse median TTP to antitumor therapy than patients with normal or at least temporary normal S-HER2 values. S-HER2 levels decreasing to values ≤15 ng/ml could be indicative for longer TTP.

402 Classification of breast disease by quantitative RT-PCR analysis of multiple transcripts in blood.

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Background: In spite of the remarkable advances in the diagnosis and treatment of breast cancer, the prognosis for patients with advanced disease is poor and the 5-year survival of patients with metastatic disease is still less than 22%. The metastatic potential of tumors is still determined by histology, a relatively invasive and subjective procedure. To identify new methods for early detection and determination of the metastatic potential of breast disease, we measured the blood levels of 8 selected genes with the aim of assessing their independent or combined sensitivity and selectivity in the classification of breast disease.

Materials and Methods: Venous blood was collected into blood-RNA tubes from 34 fully informed and consenting patients and classified as i) at risk for the development of breast cancer based upon the Gail Model (n=9), ii) with benign disease (n = 10), or iii) with breast cancer (n=15). Total RNA was isolated and examined by denaturing agarose-formaldehyde electrophoresis to confirm quality before analysis of equal amounts of total RNA from each donor by quantitative real-time polymerase chain reaction (qRT-PCR). Oligonucleotide primers specific for HER2/neu, topoisomerase II α (TOPOIIα), matrix metalloproteinase 2 and 9 (MMP2 and MMP9), vascular endothelial growth factor (VEGF), tissue inhibitor of metalloproteinase 2 (TIMP2), cytokeratin 19 (CK19), NM23-H1 and β-actin were utilized in a standard qRT-PCR reaction. All results were normalized with β-actin and analyzed using different clustering algorithms and principal component analysis.

Results: The transcript levels for MMP2 were lower and those for NM23H1 were higher in all the breast cancer categories examined. MMP9 transcript level was significantly different in high-risk patients as compared to patients with benign disease and breast cancer (p=0.048 and p=0.007, respectively). The level of each transcript examined was different in each group, but the difference within each group was not significant. Hierarchal and K-mean clustering reveals some relationships and clustering characteristic of different categories of breast disease.

Discussion: The early identification of patients with breast cancer and accurate classification of disease status is crucial for the institution of aggressive and timely therapy that can increase patient survival. A blood-based system by which these patients can be identified preoperatively would enable this. Our preliminary results show that simultaneous screening for multiple transcripts provides relatively more information that may be clinically useful in the classification of breast disease via a minimally invasive procedure.

403 PDEF, an Ets family transcription factor overexpressed in breast tumors and breast cancer patient blood samples.

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PDEF belongs to the ETS family of transcription factors, essential regulators of genetic programs for multiple cellular functions in cancer. Expressed almost exclusively in the normal prostate, lower levels of PDEF are observed in the mammary gland, and colon. We have discovered that most breast tumor cell lines express greater amounts of PDEF than normal breast tissue and/or cell-lines suggesting that it may be involved in breast cancer initiation and/or progression. Furthermore, PDEF is undetectable by RT-PCR amplification and Northern blot analysis in peripheral blood lymphocytes and lymphocytic cell lines, indicating that it can be used as a target for detection of circulating tumor cells in blood samples or lymph nodes. In order to detect extremely low levels of PDEF expression we used "realtime" RT-PCR on RNA extracted from breast tumors, breast cell-lines, patient blood samples and normal tissues (Clontech) using using a LightCycler (Roche) machine to measure fluorescent signal at the end of the elongation step on a cycle-by-cycle basis leading to real-time monitoring of PCR products. Conversion of cycle number into mRNA concentration was done from a standard curve generated by using the pCRII-PDEF plasmid at different concentrations as PCR templates in parallel with the sample reactions. To avoid amplification of contaminating genomic DNA, the genomic structure of the human PDEF gene was determined bioinformatically and primers were designed to amplify a 129 bp fragment encompassing the splice junctions of exon IV and V with exon III.

High levels of PDEF were found in breast tumors and the breast tumor cell lines MDA-MB-468, HTB-25, and SKBR3, as well as in the prostate tumor derived cell line PC3. Lower levels of PDEF are seen in the breast cell line MCF7 and HTB-26. Both normal colon and breast tissue had much lower levels of PDEF mRNA. Neither the normal breast cell line 184, the T-cell cancer cell line CEM, normal liver, nor human fibroblasts had detectable levels of PDEF mRNA expression. PDEF mRNA was detected in the blood of patients with node positive disease, and with distant metastases, but not in patients with benign breast disease. The correlation between the detection of PDEF mRNA in blood and established histopathological parameters in breast cancer patients will be presented.

404 Breast cancer specific gene-1 (BCSG1): an additional arm in the triple assessment of breast cancer diagnosis?

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Background:The diagnosis of DCIS in patients confers a much better prognosis than an invasive cancer. However, up to 28% of previously diagnosed DCIS is relabelled invasive cancer when reviewed by another pathologist, at a later date. This ethically approved study examined the expression of BCSG1, which is part of a family of genes that is breast specific, in both breast cancer specimens and lymph nodes.

Methods:28 paired tissue specimens, i.e. one tumour sample and one background sample, from 14 patients with varying stages of breast cancer, ranging from DCIS to highly invasive disease were obtained and subjected to investigation with BCSG1. In addition, 19 lymph nodes were also investigated in the same manner. All the lymph node samples had their status confirmed histologically prior to entry into the study; 10 were node-positive and 9 node-negative.

Results:Of our 14 patients, 7 had DCIS, 4 had invasive disease with minimal nodal involvement and a further 3 had highly aggressive metastatic disease. BCSG1 was expressed in 3 of the 14 background samples; partially expressed in 4 of the 7 DCIS samples and greatly expressed in 3 of the 4 highly invasive cancers. All 10 node- positive samples expressed BCSG1, while 2 of the node-negative group showed BCSG1 expression. The results have undergone statistical analysis and are shown to be statistically significant. Conclusions:BCSG1 is a breast specific gene that is highly expressed in breast cancer. It has the potential to be a useful adjunct in the diagnosis of the disease, as well as in the detection of micrometastatic deposits.

405 The correlation of CEA and CA15.3 with the extent of breast cancer using ^{18}fluorodeoxyglucose positron emission tomography.

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Background
Tumor markers (CEA and CA15.3) are frequently used to monitor breast cancer after primary treatment. The value of these markers is still not fully elucidated. Positron emission tomography (PET) has been more widely used to detect or confirm metastasis in cancer patients. Total lesion glycolysis volume in PET scanning reflects the total tumor burden. Our study aims to determine firstly, whether CEA and CA15.3 correlates with the total tumor burden. Secondly aims to identify the site of metastasis according to the tumor marker level.
Method
Breast cancer patients who have had ^{18}FDG-PET during the period of January 2000 to April 2002 were reviewed. There were a total of 41 patients and a total of 61 PET scans performed. CEA and CA 15.3 were taken at the time of the tomography. The values were then correlated with the total lesion glycolysis volume (sum of all lesion volume x SUVmax of lesion) and the sites of metastasis by bivariate correlation. The differences in mean values of these tumor markers were determined by independent t-tests.
Results
CEA and CA15.3 correlated positively with total glycolysis volume. However, only CA15.3 reached statistical significance (correlation coefficient = 0.820; $p<0.001$). CA15.3 also correlated significantly with the number of bone metastasis (correlation coefficient = 0.327; $p = 0.022$). The mean value of CA15.3 for patients with bone metastasis was 303.6 U/ml and for those without bone metastasis was 24.5 U/ml. Although CEA correlated positively with the number of liver metastasis, the difference was not statistically significant.
Conclusion
CA15.3 is useful in predicting the total tumor burden in breast cancer. Elevated CA15.3 level is a strong and good predictor of bone metastasis in breast cancer.

406 Prospective evaluation of CEA and CA 15.3 in patients with locoregional breast cancer.

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Tumor markers were prospectively studied in the sera of 1200 untreated patients with breast cancer diagnosed between 1982 and 2001. Abnormal CEA and CA 15.3 serum levels were found in 14%, and 17% of patients, respectively. Both tumor markers were correlated with tumor size, TNM and nodal involvement. CEA was also related to menopausal status, and ER. Univariate analysis (mean follow-up 12 years) show that CEA and CA 15.3 were prognostic factors with significantly shorter disease free survival (DFS) and overall survival (OS) in patients with pretreatment tumor marker positivity. Multivariate analysis (1026 patients) in DFS and in OS show that nodal involvement, CEA, ER, adjuvant treatment but not Tumor size, menopausal status, histological grade, histology, CA 15.3, PgR, p53 (345 patients) or c-erbB-2(345 patients) in tissue are independent prognostic factors. Multivariate analysis in node positive and in node negative patients show that tumor size, ER, menopausal status, CEA (node positive) and CA 15.3 (node negative) are independent prognostic factors. In summary, tumor markers are an useful, cheap and reproducible tool in prognosis.

407 Matrix metalloproteinase 2 and 9 activity in blood of patients with breast disease and at risk for development of breast disease.

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Background: Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which play significant roles in extracellular matrix modification and angiogenesis. MMPs occur as inactive pro-enzymes, active enzymes and enzyme inhibitor complexes and are differentially regulated. Two members of this family, MMP2 and MMP9, have been widely implicated in breast cancer invasion and metastasis. It is however, not known if changes in the blood levels of MMP2 and/or MMP9 has any clinical significance. In this study, we describe the activity of MMP2 and MMP9 in blood of patients with breast disease and at risk for development of breast disease. The aim was to determine if such differences could be used to stratify breast disease or predict the risk for the development of breast cancer.
Materials and Methods: Blood was obtained from 75 fully informed and consenting donors, consisting of patients at risk for the development of breast cancer (n=17), patients with benign breast disease (n=32) and patients with breast carcinoma (n=26). Each blood specimen was screened for MMP2 and MMP9 activities using an activity assay method that quantitatively measures active and total (active and activatable) forms of each metalloproteinase. Data obtained were collated and analyzed to determine the statistical significance of the results obtained.
Results and Discussion: Total MMP9 was significantly higher in the high risk group ($p=0.004$) while active MMP2 was significantly lower in carcinoma patients ($p< 0.002$). Multivariate analysis of active and total levels of both MMPs revealed disease-state dependent clustering. This suggests that the measurement of MMP2 and MMP9 activities in blood may facilitate the early detection of breast disease or classification of disease progression via a minimally invasive process.

408 Urinary deoxypyridinoline for detection of bone metastasis in Taiwanese women with breast cancer.

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Background: Urinary deoxypyridinolive (Dpd), a crosslink product of collagen molecules found in bone and excreted in urine during bone degradation, was recently described as a marker of bone turnover in metastatic breast cancer.
Materials & Methods: In this study, the urinary deoxypyridinoline / creatinine (Dpd/Cre) ratio was determined using enzyme immunoassay in the samples from 116 women with metastatic breast cancer. Bone metastases were confirmed by bone scan and/ or x-ray or CT scan, and follow-up over six months.
Results: The ratios of Dpd/Cre were significantly higher in patients with bone metastases than in those without bone metastasis ($P<0.05$). In patients with bone metastasis, significantly higher ratios of Dpd/Cre were observed in those with multiple lesions than in those with a solitary lesion, and these values also reflected therapeutic response ($P<0.05$). Serial monitoring of Dpd/Cre revealed that this elevation was correlated with disease progression. Patients with stable bone disease under effective therapy had a significant fall of the Dpd/Cre ratios in comparison to the progressive group ($P<0.05$).
Conclusion: Monitoring of Dpd/Cre ratios is helpful in providing additional information for detection of bone metastasis and evaluation of therapeutic response to bone metastasis. Urinary Dpd/Cre ratios may be a useful bone markers for detecting bone metastasis and evaluating the therapeutic response.

409 Impact of tamoxifen on the risk of metachronous contralateral breast cancer in women with germline mutations of BRCA1 or BRCA2.

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Background: Women with hereditary breast cancer due to germline mutations of BRCA1 or BRCA2 are known to be at high risk for second primary cancers, especially contralateral breast cancer (CBC). One case-control study has indicated that the risk of CBC is reduced in mutation carriers taking tamoxifen, but a subset analysis of the Breast Cancer Prevention Trial did not clearly indicate such a benefit.

Subjects and Methods: A retrospective anonymized design was employed. Women of Jewish religious preference undergoing breast conservation therapy for invasive breast cancer between 1980 and 1990 were identified from review of clinical databases. Paraffin-embedded tissue and follow-up information was available for 314 women, 305 of whom were successfully genotyped, after anonymization, for the Ashkenazi founder mutations BRCA1 185delAG and 5382insC, and the BRCA2 founder mutation 6174delT. Clinical outcomes in women with or without mutation have previously been reported (JNCI 1999;91:2112-2117). In this report, we describe the impact of tamoxifen on CBC risk in this population.

Results: Germline mutations were detected in 28 women (9.2%). Information regarding tamoxifen use was available for 25 women. Of 5 mutation carriers taking tamoxifen, 1 developed CBC, compared to 6 of 20 mutation carriers not taking tamoxifen. The hazard ratio for CBC among tamoxifen users compared to non-users was 0.57 [95% CI: 0.07-4.57; P=0.6]

Conclusions: In this small series, tamoxifen appeared to decrease the risk of CBC among mutation carriers, but this reduction was not statistically significant. Expansion of the dataset is continuing.

Supported by DAMD17-01-1-0325.

410 Classification of hereditary and sporadic breast cancers based on clinicopathological data.

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Intro: About 5% of all the breast cancer cases are due to an inherited predisposition by germ line mutations in the BRCA1/2 genes. Screening for BRCA mutation in suspected carriers is however difficult and mutations may well be missed, which hampers genetic counselling. Additional features pointing to hereditary breast cancer are therefore useful.

Methods: Age of presentation, tumour size, mitotic index (MAI) and the expression of Ki67, p53, p21, p27, cyclin D1, cyclin A, estrogen (ER) progesterone (PR), Epidermal Growth Factor (EGFR), and HER-2/neu receptors by immunohistochemistry were compared between 27 proven BRCA1/2 mutation carriers and over 500 unselected controls. In addition, a limited number of cases at intermediate risk of hereditary disease based on familial history were evaluated.

Results: Hereditary breast cancers showed higher MAI, and Ki67/p53/cyclin A indices, more frequent EGFR overexpression, and lower ER/PR compared to the controls. Based on a discriminant function including age, Ki67 and EGFR, 96% of the hereditary cases and 92% of the control cases were correctly classified. Most cases at intermediate risk of hereditary disease based on familial history could be classified with high propability as either hereditary or sporadic with this classification function.

Conclusion: Breast carcinomas can be classified as sporadic or as part of a hereditary syndrome with a high level of certainty using a discriminant function based on age, Ki67 and EGFR. This could be clinically useful to guide mutation analysis in families with borderline risk of hereditary disease.

411 Intensified surveillance and prophylactic surgery for breast and ovarian cancer: acceptance and participation among high-risk women in southern Germany.

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Background: Women with a hereditary predisposition for breast and ovarian cancer carry a significantly increased risk of developing these cancers. Mutations in BRCA1/2 account for the majority of the familial cases and confer a lifetime risk of up to 80% for breast cancer and up to 60% for ovarian cancer. **Objective:** To assess acceptance and participation of women at increased risk for breast and ovarian cancer regarding available management options: 1) intensified surveillance programs, 2) prophylactic surgery. **Materials and Methods:** Questionnaires were mailed to 168 high-risk women, who had been counselled for genetic testing and preventive strategies at our hereditary cancer clinic due to an estimated lifetime risk for breast or ovarian cancer of at least 20% based on family history. None had a personal history of breast or ovarian cancer. The questionnaires collected information concerning 1) participation in the recommended surveillance program, which comprises regular clinical breast and pelvic exams, mammograms, breast-MRIs, breast and transvaginal ultrasounds and CA125 measurements and 2) intention to undergo prophylactic surgery. **Results:** Of the 168 women surveyed, 84 (50%) responded: Five percent were BRCA1/2 positive, 10% were carrying an unclassified variant (UV), 33% were BRCA1/2 negative, for 32% the test result was pending, 8% had declined genetic testing and 12% responded anonymously, not stating their BRCA1/2-status. All mutation-carriers (100%) and 88% of UV-carriers were participating in intensified surveillance. Of the remaining responding women 35% had chosen intensified surveillance. Five women (6%) had undergone prophylactic oophorectomy or mastectomy, two being BRCA1/2 positive, two being negative and one carrying an UV. Of the remaining surveillance-participants 34% considered prophylactic surgery as a management option compared to 21% of the not participating group. **Discussion**: The acceptance to participate in intensified surveillance increases clearly with a known mutation or UV in BRCA1/2 and is paralleled by a greater acceptance of prophylactic surgery. Knowing that women with a strong family history but a negative, pending or declined BRCA1/2 test are also at high risk for developing cancer, counselling towards participating in surveillance programs has to be emphasized.

412 Risk perception in women at increased risk for breast cancer (BC) due to a family history or a genetic predisposition.

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Background: After genetic counseling there is often a lack of concordance between actual risk and perception of risk of developing BC. More insight into this phenomenon may be of help for genetic counselors and other involved clinicians.

Objective: To determine accuracy of risk perception and association with psychological factors in women at increased risk for BC, participating in a nationwide surveillance program (MRISC study).

Material and Methods: Participants are classified into three objective risk categories implying a cumulative life time risk (CLTR) of developing BC. Category 1 implies a CLTR of 60-85% (BRCA mutationcarriers), cat. 2: 30-50%, and cat. 3: 15-30%. Two months prior to their surveillance appointment participants complete a questionnaire measuring knowledge of their risk of developing BC, psychological factors and demographics.

Results: 241 women completed the questionnaire; 27 women of cat. 1, 128 of cat. 2 and 86 of cat. 3 (mean age 40 yrs). Demographic variables did not differ between the risk categories, however the amount of years adhering to surveillance did (3 yrs., 5 yrs., 6 yrs. respectively). Logistic regression analysis, adjusted for demographic variables, of the accuracy of risk perception reveals a trend towards difference between the risk categories (p=.06). Of the women in cat. 3 only 32% had an accurate risk perception, in contrast with 70% in cat. 1. With regard to educational level there was a positive trend towards difference (p=.067).

Using Discriminant Analysis we explored psychological variables associated with over- and underestimation of risk. A significant association was found with intrusion, meaning overestimation of risk (predominantly found in cat. 3) relates to being overwhelmed with involuntary feelings and thoughts about BC.

Conclusion: Accuracy of risk perception of developing BC is related to the objective risk status of a woman and the educational level.

Women overestimating their risk of developing BC are more frequently overwhelmed by involuntary thoughts and feelings about BC. Repeated measures should reveal the impact of risk perception on wellbeing, adjustment and adherence to surveillance.

413 Factor V Leiden (FVL) and prothrombin G20210A (PTG) mutations and risk of thromboembolic events (TE) in NSABP P-1, the breast cancer prevention trial (BCPT).

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Background: Tamoxifen (T) therapy has been associated with an increased risk of thromboembolic events (TE) in the treatment of women with breast cancer and in healthy women. In the BCPT, RR of DVT among women on T was 1.60 (95% CI=0.91-2.86) and of PE was 3.01 (95% CI=1.15-9.27). FVL and PTG are germline mutations in clotting factors associated with increased risk of TE with oral contraceptives, pregnancy and other hormonally enhanced states. If risk of clotting on T were attributable to FVL or PTG, then clinical testing for these mutations before T use could alter therapeutic decisions.

Methods: A nested matched case-control design was used. Cases were 77 BCPT subjects with a PE or DVT during study participation. 299 Controls (4:1 per case) were selected at random from subpopulations of nondiseased participants matched to cases on age at entry (+3 years), race, treatment (T v. placebo), smoking status at entry, and duration of treatment at time of clot (+ 3 months). Anonymized samples were analyzed for FVL and PTG mutations using standard multiplex PCR-based techniques.

Results: No significant differences were observed between cases and controls on matching variables, aspirin use, or diabetes history. Mean Body Mass Index (BMI) was 32.1 in cases v. 27.2 among controls ($p<0.001$). 29 mutations were found: 9 in cases, 20 in controls: 18 FVL, 8 PTG, 3 both: 16 were in placebo, 13 in the T group. OR for TE with mutation 1.90 (95% CI 0.72-4.76); OR for TE with mutation adjusted for BMI.2.34 (95% CI 0.86-5.99). No interaction between T and mutation status and risk of TE was observed.

Discussion: In the BCPT dataset, risk of TE associated with T therapy is about 2-fold and appears within 6 months of initiation of T; BMI is also significantly associated with TE. Neither FVL nor PTG mutations appear to interact with T to modify the risk of TE.

414 Proof of principle: mammographic density reduced by a gonadotropin-releasing hormone agonist (GnRHA)-based chemoprevention regimen for young women at high risk for breast cancer.

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Mastectomy and oophorectomy are surgical options for management of women with germline mutations in *BRCA* genes, which 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. Oophorectomy reduces breast cancer risk in *BRCA* mutation carriers, but has an irreversible impact on fertility. Menopausal symptoms and concerns about impaired fertility and safety limit the utility of SERMs as chemopreventives in young women. The purpose of this study was 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. It is predicted that such a regimen will reduce lifetime breast cancer risk by one third if used for 5 years and by more than 70% if used for 15 years. Results from this study of young women with high breast cancer risk indicate that deslorelin clearly induced a substantial reduction in mammographic densities, as measured by previously reported qualitative and quantitative methodology. 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 60 days), and quality of life is preserved. The regimen reduces mammographic density and presumably breast cell proliferation, making it a promising non-surgical risk reduction option. Thus, the regimen may not only result in enhanced mammographic screening (and facilitate detection of asymptomatic cancers), but is likely to significantly reduce the risk of breast cancer and ovarian cancer. Further, deslorelin can be combined with E2 and T in a nasal spray at doses sufficient to maintain good health while achieving decreased breast density.

415 Comparison of the effects of exemestane, 17-hydroexemestane and letrozole on bone and lipid metabolism in the ovariectomized rat.

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Research Objective and Rationale: The irreversible steroidal aromatase inhibitor exemestane (EXE) and the reversible imidazole, letrozole (LET), are third generation agents currently prescribed for breast cancer patients. The effects on bone and lipid metabolism in ovariectomized (OVX) rats of EXE, its principal metabolite 17-hydroexemestane (17-hydro-EXE) and LET were studied.

Methods: OVX rats were treated by weekly intramuscular injection with 20, 50 and 100 mg/kg of EXE, 20 mg/kg of 17-hydro-EXE, and daily oral gavage with 1 mg/kg of LET for 16 weeks. At the end of treatment, bone mineral density (BMD) of the femur and whole lumbar spine was determined by dual energy x-ray absorptiometry. Bone resorption biomarker-serum pyridinoline (Pyd) and bone formation biomarker-serum osteocalcin were measured by EIA kits. Total serum cholesterol and low-density lipoprotein (LDL) were determined using an ADVIA® 1650 chemistry system analyzer.

Results: After 16 weeks of treatment; 1) lumbar spine BMD was 7.4%-14.8% and 14.0%, higher in OVX animals given 20-100 mg/kg of EXE and 20 mg/kg of 17-hydro-EXE, respectively, than in OVX controls (all $p < 0.02$). Similar effects were observed on femoral BMD. 2) EXE (20-100 mg/kg) and 17-hydro-EXE (20 mg/kg) reduced an OVX-induced increase of Pyd by 85%-97% and 95%, respectively (all $p < 0.05$), suggesting prevention of bone resorption by EXE and 17-hydro-EXE. 3) The OVX-induced increase of osteocalcin was completely prevented by treatment with EXE and 17-hydro-EXE. 4) The administration of 20-100 mg/kg EXE and 20 mg/kg 17-hydro-EXE to OVX rats caused a 22%-42% and 38%, respectively, inhibition of serum cholesterol levels (all $P < 0.002$), and reduced LDL by over 68% compared with OVX controls (all $p < 0.02$). In contrast castrated animals treated with LET had BMD, bone biomarkers and lipid levels similar to control OVX rats.

Conclusion: The data show EXE and its principal rodent and human metabolite 17-hydro-EXE significantly prevent bone loss and lower serum cholesterol and LDL levels in OVX rats. These protective effects on end-organ function are not seen with the non-steroidal inhibitor LET and are of potential clinical value.

416 All-trans-retinoic acid but not tamoxifen induces CBP and p300 expression in human mammary epithelial cells: a rationale for breast cancer chemoprevention.

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CBP and p300 are coactivators of estrogen- and retinoid-signaling, regulate proliferation and apoptosis, and are hypothesized to play and important role in BRCA1-mediated transcription-coupled DNA repair. The transcriptional regulation of CBP and p300 themselves is poorly understood. All-trans-retinoic acid but not tamoxifen induced CPB/p300 mRNA and protein expression in normal human mammary epithelial cells (HMECs). To assess whether CBP and p300 are regulated by retinoic acid receptors (RARs) two systems were tested: 1) ATRA-resistant MCF-7 breast cancer cells were transduced with a functional RAR-beta2 and 2) human mammary epithelial cells (HMECs) were transduced with the dominant negative RAR, RARalpha403. Expression of RAR-beta2 in MCF-7 cells resulted in increased sensitivity to ATRA and increased CBP/p300 protein and mRNA levels upon ATRA treatment. Inhibition of RAR function in HMECs resulted in resistance to ATRA, decreased CBP and p300 protein and mRNA levels, and loss of CBP and p300 induction upon ATRA treatment. Suppression of CBP or p300 in HMECs resulted in a loss of sensitivity to ATRA. Loss of ATRA-mediated growth inhibition and decreased transactivation of a RARE-driven CAT reporter were observed. CBP and p300 are normally present in limiting amounts and are recruited during ATRA mediated transcriptional regulation, their regulation by ATRA and RARs may be an important element in transcriptional control of ATRA regulated gene expression in HMECs. CBP and p300 are required for growth regulation and apoptosis and can be downregulated in cancer. Therefore, a means of upregulating CBP and p300 by retinoids may be an useful adjunct in the prevention of breast cancer, particularly in BRCA1-mutation carriers.

417 A phase 2 trial of raloxifene in premenopausal women at high risk for developing invasive breast cancer.

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We conducted an open-label, uncontrolled, phase 2 trial to study the safety of raloxifene hydrochloride, 60 mg/day, in premenopausal women at high risk of developing invasive breast cancer. Raloxifene, a selective estrogen receptor modulator, is approved for prevention and treatment of postmenopausal osteoporosis. A primary objective was to determine the change in anterior-posterior (AP) spine bone mineral density (BMD). Additional objectives included assessment of endogenous hormones and lipids, menstrual cycle changes, and endometrial and ovarian effects. Premenopausal women (ages 23 to 47) at increased risk by virtue of: Gail model risk > 1.7% over 5 years; lobular neoplasia, ductal carcinoma in situ, BRCA1/2 mutation positive, or a very strong family history were eligible. Because raloxifene is contraindicated in women who are or may become pregnant due to possible teratogenic effects, all women were required to use non-hormonal birth control for the duration of the study and for 3 months after completion. Raloxifene 60 mg was administered daily for 2 years. To date, 31 women have enrolled. An interim analysis was conducted after 12 women completed 1 year of raloxifene. The average AP spine BMD change from baseline at 1 year was -1.74 % (p=0.03, 95% C.I. -3.3 to -0.2). Adverse events have been mild, including menstrual irregularities, hot flashes, leg cramps, and mood lability. These preliminary data suggest that raloxifene 60 mg/day may be well-tolerated in premenopausal women. Although conclusions cannot be drawn from this small sample, the observed reduction in BMD is consistent with the reported effect of tamoxifen on BMD in premenopausal women. The change in BMD and adverse events observed suggest raloxifene 60 mg/day has biological activity in this population. Additional studies are needed to determine whether raloxifene use by premenopausal women is safe and effective in reducing the risk of invasive breast cancer.

418 The selective estrogen receptor modulator SCH 57068 prevents bone loss, reduces serum cholesterol and blocks estrogen-induced uterine hypertrophy in the ovariectomized rat.

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Research Objective and Rationale: We are investigating whether the new selective estrogen receptor modulator SCH 57068 can replace the progestin component of standard hormone replacement therapy (HRT) to achieve a better therapeutic index. We have thus investigated the effects of SCH 57068 alone and with estradiol (E_2) on bone, lipids and uteri in ovariectomized (OVX) rats.

Methods: Rats OVX with and without E_2 were treated by oral gavage for 12 weeks with daily doses (0.01, 1, 2.5 mg/kg) of SCH 57068. At the end of treatment, bone mineral density (BMD) of the femur and whole lumbar spine was determined by dual energy x-ray absorptiometry. Bone resorption biomarker-serum pyridinoline (Pyd) and bone formation biomarker-serum osteocalcin were measured by EIA kits. Serum cholesterol and low-density lipoprotein (LDL) were determined using an ADVIA® 1650 chemistry system analyzer.

Results: After 12 weeks of treatment, 1) lumbar spine BMD was 8.8-9.4% higher in OVX animals given SCH 57068 than in OVX controls (all $p < 0.005$). Similar effects were observed on femoral BMD. 2) SCH 57068 reduced an OVX-induced increase of Pyd by 91-93% (all $p < 0.02$), suggesting prevention of bone resorption by SCH 57068. 3) The OVX-induced increase of osteocalcin was completely prevented by treatment with SCH 57068. 4) The administration of 0.01-2.5 mg/kg SCH 57068 to OVX rats caused 41-42% inhibition of serum cholesterol levels (all $p < 0.0001$), and reduced LDL by over 95% (all $p < 0.05$). 5) No stimulatory effect of SCH 57068 was observed on the uterine weight and endometrium at any doses. SCH 57068 (1 and 2.5 mg/kg) reduced the E_2-induced increase of uterine weight by 42% and 55% (all $p < 0.0001$), respectively. 6) SCH 57068 reduced weight gain in OVX rats by 63-69% (all $p < 0.0001$).

Conclusion: SCH 57068 prevents OVX-induced bone loss and elevation of serum cholesterol and LDL levels, and blocks E_2 stimulation of the uterus. The results suggest that SCH 57068 could be used to replace the progestin component of HRT and the combination of SCH 57068 plus E_2 could be a novel HRT with breast cancer preventative effects.

419 Tamoxifen may not just prevent ER positive cancers: an alternate hypothesis.

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Introduction: The data from the NSABP P01 trial and the Multiple Outcomes of Raloxifene (MORE) Trial strongly suggest that Tamoxifen (Tam) reduces the risk of estrogen receptor positive (ER+) tumors only. Described mechanisms of Tam resistance, including loss of ER expression and induction of co-activators, may, exist de novo. It has also been reported that ER+ primary tumors can recur as ER- in 20% of cases. Finally, data from BRCA 1 and 2 carriers who undergo oophorectomy show that there is an equal reduction (60%) in risk of BRCA1 tumors (primarily ER-) and BRCA2 tumors (primarily ER+). The conditional probabilities from pooled NSABP trials B18, B22 and B25(Swain et. al.,ASCO Proceedings 2002), show that a woman with an initial ER + tumor had a much less likely chance of having an ER+ tumor if she was >50 and received Tam, than if she was <50 and did not (57% vs. 86%). However, if the first cancer is ER-, the likelihoods are 72% and 80% that the second cancer will be ER-, for women <50 and >50yrs, respectively. The explanation provided is that Tam reduces what would have become ER + cancers. However, the ER status of the first cancers strongly predict the ER status of the subsequent cancer when Tam is not given. This suggests that Tamoxifen exposure could result in some tumors originally destined to be ER positive to appear as ER negative. Using data from the PO1 and MORE studies, we propose an alternative explanation for the observed results of prevention trial.

Methods: Actual cancers from the NSABP P-01 and MORE studies were used to investigate the possibility that Tam equally reduces the emergence of ER - and ER+ tumors, but promotes the conversion of a small fraction of ER+ to ER -, resulting in the same observed data. Assuming that some proportion, z, of ER+ tumors become ER negative after exposure to Tamoxifen and there is an equal reduction in risk of both ER- and + tumors, we solved for the conversion rate, and the risk reduction rate.

Results: At a conversion rate of z = 20% of ER+ to ER- tumors (with Tamoxifen exposure) and equal reduction of risk for both ER- and ER+ tumors at a level of 60%, the outcomes of the PO1 Tam-treated group can be predicted. Solving the equation again, using the MORE data, an almost identical result is found: 23% conversion rate and a very similar reduction in risk of 58%.

Conclusion: An alternate hypothesis of how Tam affects emerging tumors is presented. It is entirely possible that Tam influences conversion of some incipient tumors to become ER-, leaving Tam to affect a 60% risk reduction in ER- and + tumors, similar to the risk reduction seen in high risk women undergoing oophorectomy. This hypothesis suggests that we should develop and assess possible markers Tam resistance to avoid harm in the prevention setting.

420 Chemopreventive effects of rosiglitazone, 9-cis-retinoic acid and targretin in the methylnitrosourea (MNU) induced model of mammary cancer in rats.

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The chemopreventive effects of various agents administered singly or in combination were examined in a rat model which develops multiple hormonally responsive mammary cancers. Female Sprague-Dawley rats (50 days old) were administered a single IV injection of MNU (50 mg/kg BW). Beginning 5 days later, rats were administered the PPAR gamma agonist rosiglitazone {ROS} [20 or 60 mg/kg BW/day] by gavage or the RAR/RXR pan agonist 9-cis retinoic acid {9cRA} [80 mg/kg diet]. ROS and/or 9cRA were given alone or in combinations for the duration of the experiment (120 days post MNU). Control, ROS (Hi Dose), ROS (Lo Dose) and 9cRA treated rats developed 4.2, 2.5, 3.6, and 1.5 mammary tumors per rat, respectively. Interestingly, the Hi Dose of ROS resulted in the formation of lipomas in almost 100% of rats during the study. Somewhat surprisingly, the combination of ROS plus 9cRA, which should favor the formation of heterodimers between the PPAR gamma and RXR nuclear receptors, was not more effective than treatment with 9cRA alone in this model. In addition, a relatively specific RXR agonist targretin (TRG) is being examined for its ability to inhibit MNU induced mammary tumors. RNA data comparing and contrasting the abilities of ROS, 9cRA and TRG to modulate gene expression in mammary tumors from this model shall also be presented.

421 Evaluation of DFMO effects in breast cancer chemoprevention study.

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Background: D,L-difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (ODC), has shown anticancer activity in vitro and in vivo. This study tested the hypothesis that DFMO effectiveness is mediated through downregulation of proteins that promote tumor cell invasiveness. The objective was to determine whether two weeks of DFMO therapy prior to definitive surgery decreased proliferation and invasiveness and increased apoptosis of early stage breast cancer.

Materials and Methods: The DFMO cohort (n=28) consisted of women with DCIS or T_1 or T_2 breast cancer who had a diagnostic core biopsy showing DCIS or breast cancer (pre-DFMO). They received DFMO (11 patients at 0.5g/m^2 po daily and 17 patients at 3g/m^2 po daily) for 10 to 14 days followed by definitive lumpectomy or mastectomy (post-DFMO). The control cohort (n=14) included core biopsies of women with DCIS or invasive breast cancer who gave permission to study biomarkers in their diagnostic core biopsies (pre-control) and definitive surgery tissues (post-control). Expression levels of MiB-1 (Ki-67), matrix metalloproteinases-2 and -9 (MMP-2, MMP-9), and urokinase plasminogen activator were measured in fibroblasts and tumor tissue; uPA(F) and uPA(T), respectively, were evaluated in paraffin sections from biopsy tissue by immunostaining with human-specific antibodies. Apoptosis was quantified by TUNEL assay. The number and intensity of tumor cells stained with each of the biomarkers were counted under a light microscope and compared in pre-DFMO and post-DFMO samples and in the corresponding control breast cancer tissue by the study pathologist.

Results: A decrease was noted in the expression of MMP-2 (in the DFMO and control groups) and a trend towards decreased uPa(F) and increased TUNEL activity was observed in the DFMO group. No differences in biomarker modulation were noted in the 0.5g vs 3g dose groups. The 10-14 days of DFMO treatment were well tolerated.

Discussion: The results suggest no significant quantitative differences in the expression levels of examined biomarkers of invasiveness and proliferation but a trend towards increased apoptosis and decreased uPa(F) with DFMO.

422 Effects of various doses of tamoxifen and the aromatase inhibitor vorozole on RNA expression in MNU induced mammary tumors.

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Effects of various doses of tamoxifen and the aromatase inhibitor vorozole on RNA expression in MNU Induced mammary tumors

The effect of tamoxifen and vorozole on RNA expression in hormonally responsive mammary tumors induced by methylnitrosourea (MNU) was determined. Female Sprague-Dawley rats (50 days old) were given a single i.v. injection of MNU. Beginning 5 days later, rats were given tamoxifen in the diet (0.66 and 0.13 mg/kg diet) or vorozole (0.32 mg/kg BW/day, i.g). These treatments decreased tumor multiplicity 55, 12 and 65% respectively relative to the MNU controls. At the time animals were sacrificed, tumors were collected and RNA isolated. The tumors were individually probed for RNA expression with a large Affymatrix or a smaller Clontech microarray. The overall patterns of changes and magnitude of change for RNA expression for the large array were strikingly similar for tumors from rats treated with either dose of tamoxifen despite their differences in efficacy. However, a limited number of genes displayed dose dependent differences in expression. Expression was compared between control tumors and tumors from rats treated with similarly effective doses of tamoxifen (0.66 mg/kg diet) and vorozole (0.32 mg/kg BW/day) employing a small Clontech microarray (588 genes). Interestingly, 28/588 genes were modulated by tamoxifen and 10/588 genes were modulated by vorozole. However, only a single gene (insulin growth factor binding protein 1) was modulated by both agents. These result show: 1) one can observe modulation of multiple genes in vivo by treatment with effective agents; 2) most gene changes are similar with either moderately or minimally effective doses of tamoxifen, and 3) gene changes associated with effective doses of agents from different classes generally did not overlap.

423 Longitudinal HER-2/neu measurements during treatment with Herceptin, epirubicin plus cyclophosphamide (HEC): interim serum results of a phase II study in patients with metastatic breast cancer.

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Introduction: The extracellular domain of HER-2/neu is cleaved from the membrane-bound oncoprotein by metalloproteases and is shed into the blood stream. We report of the clinical utility of monitoring serum HER-2/neu (sHER-2/neu) in metastatic breast cancer patients (pts) in a phase II safety trial using Herceptin (H), epirubicin (E) plus cyclophosphamide (C). Material/methods: A total of 353 samples were collected from 24/26 pts treated at dose level I (60 mg/m2 E). Samples were collected weekly from baseline to week 7 and every 3 weeks thereafter during HEC therapy. sHER-2/neu was measured using an FDA-approved ELISA assay (Oncogene® Science/Bayer Corporation, Cambridge, MA) with an upper limit of normal (ULN) of 15 ng/mL. Results: Baseline sHER-2/neu levels ranged from 8.7-1494 ng/mL (median: 25.4 ng/mL; mean: 138 ng/mL) while CA27.29 ranged from 14.4-2426.9 U/mL (median: 93.5 U/mL; mean 339 U/mL). A total of 18/24 pts (75%) showed increased sHER-2/neu levels >15 ng/mL at baseline. In the cohort of dose level I, 14/24 (58.3%) pts had an objective response (PR/CR). These responses were achieved in 61% of the sHER-2/neu positive pts and in 50% of the sHER-2/neu negative pts. By week 4, the sHER-2/neu levels had decreased by a mean of 138.6 ng/mL (i.e. 73.8%) in comparison to baseline in the subset of pts with PR/CR while the sHER-2/neu level in pts with progressive disease showed no relevant change. Only one pt with progressive disease showed decreasing sHER-2/neu levels by week 4. The slope of the sHER-2/neu levels was much more pronounced (decrease of 9 times ULN) than the relative changes of the CA27.29 levels with a mean decrease for responders of 59 U/mL (twice ULN). Conclusions: Pts with clinical benefit from HEC therapy show normalization of the sHER-2/neu level irrespective of the baseline concentration baseline. This decrease is more pronounced than relative changes of CA27.29 which generally do not normalize. Measurements of sHER-2/neu give predictive information on the benefit from therapy. The prompt decreases of sHER-2/neu are probably not due to reductions of tumor mass, but to changes of the shedding process by Herceptin itself.

424 Response to trastuzumab (Herceptin) given with paclitaxel (taxol, T) immediately following 4AC as initial therapy for primary breast cancer (BrCa).

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INTRO: Herceptin (H) is active in HER-2 overexpressing (H2+) metastatic BrCa, and synergizes with some chemotherapy (ChRx). The impact of adding H to the adjuvant ChRx 4AC (doxorubicin/cyclophosphamide) followed by T is not known. Neoadjuvant ChRx allows assessment of response, a valuable surrogate marker for prognosis. In a prospective phase II institutional trial, we assessed clinical and pathologic response rates (RR) to neoadj 4AC followed by wkly T+H followed by surgery for untreated high risk H2+ BrCa patients (pts), and compared these RR to a cohort treated with 4AC followed by single agent q 3 wk T.

METHODS: We treated 39 stage IIb, III, and presenting stage IV pts. We assessed clinical RR after 4AC and again after T, and pathologic RR after all ChRx at surgery. 22 H2+ pts received 4AC (60/600 mg/m2 iv q 3 wks x 4) followed by weekly T+H (T 90mg/m2 + H 2mg/kg x 12 wks). 17 pts in the non-randomized comparison group (H2-negative or declining H) received 4AC, then T alone at 175 mg/m2 iv q 3 wk.

RESULTS: Most pts (82%) are Stage III or presenting Stage IV, with 9 inflammatory cancers. More clinical and pathologic CR were seen in the 4AC+T+H pts (32%, 22%, respectively) compared to the 4AC+T pts (24%, 6%). The clinical RR (complete response CR + partial response PR) after 4AC for all 39 pts was only 51%; this rose to 77% after T+H, and also to 77% after T alone. 4AC+T+H has been well tolerated; clinical cardiotoxicity has been minimal (1 grade 3 atrial fibrillation; 1 grade 1 edema; Carey LA et al, Proc ASCO 2002).

Table 1. Pathologic stage after neoadjuvant therapy.

Stage (breast/LN):	0	I	II	III
4AC-T (n=16)	1 (6%)	2 (13%)	9 (56%)	4 (25%)
4AC-T+H (n=18)	4 (22%)	4 (22%)	8 (45%)	2 (11%)

Stage 0 = no invasive cancer; I = T1N0; II = T1-2N1, T3N0, III = T3N1, any T4. 5 of 39 patients did not have surgery.

Pretreatment HER-2 gene amplification is available on 11 of the 20 4 AC+T+H pts on whom RR to the T+H regimen is measurable. 3/6 FISH-positive pts had clinical CR to T+H compared with 1/5 FISH-negative pts.

CONCLUSIONS: In this pilot trial with ongoing accrual, 4AC followed by weekly paclitaxel plus Herceptin for 12 weeks appears to provide additional complete response benefit over 4AC alone or 4AC followed by single agent paclitaxel given every three weeks.

425 Previous cumulative anthracycline dose is the main determinant of LVEF decrease in breast cancer patients treated with trastuzumab (Herceptin®): results of a French compassionate use program.

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Introduction: Potential cardiotoxicity is the main treatment limiting adverse event of trastuzumab. We analyzed Left Ventricular Ejection Fraction (LVEF) in trastuzumab-treated anthracycline/taxane pretreated HER2/neu 2+/3+ metastatic breast cancer patients (pts) from a subset of a French compassionate use program prior to market approval (retrospectively source reviewed by independent oncologists). **Treatment:** 90 min 4 mg/kg trastuzumab infusion followed by weekly 30 min, 2 mg/kg infusions, ± a cytotoxic agent was recommended. **Results:** 133 pts treated (34 centers) from 01/99-12/00, 118 evaluated for LVEF by cardiac scintigraphy and/or echography and cardiac symptoms. Grade 1-3 cardiac events (NCI-CTC): 42 pts (36%); asymptomatic LVEF decrease: 34 pts (81%; including 6 echographic cardiac changes); symptomatic LVEF decrease: 4 pts (1 cardiac failure, 2 arrhythmia, 1 angina); tachycardia without LVEF decrease: 4 pts. No cardiac deaths occurred. Standard demographic disease/pretreatment variables were evaluated. 99% pts had prior adjuvant or palliative anthracyclines.

	Maximum grade of cardiac event/patient (NCI-CTC)		
	0	1	2-3
N pts (%) (N=118)	76 (64%)	23 (20%)	19 (16%)
Median cumulative prior doxorubicin equivalent dose (range)*	265 (60-739)	353 (0-945)	388 (176-739)
Median total duration of Herceptin treatment in weeks (range)	20 (1-101)	28 (2-121)	23 (5-97)
Median % decrease LVEF at nadir (range)	-4% (-9% to 9%)	-11% (-19% to -6%)	-20% (-47% to -9%)

* p=0.014 gr 0 vs 1-3 and p=0.01 gr 0-1 vs 2-3

Only prior cumulative anthracycline dose correlated significantly with incidence/severity of LVEF changes and/or cardiac events. 11% pts discontinued treatment for cardiac events, 7% for asymptomatic LVEF decrease (median: 10; range: 2-41). **Conclusion:** In this series, LVEF decrease likelihood in trastuzumab-treated pts correlates with cumulative prior anthracycline dose. Prospective studies are needed to validate the present retrospective analysis.

426 Serum HER-2/neu extracellular domain parallels responses to trastuzumab treatment of recurrent breast cancer.

Pichon MF, Neumann R, Guepratte S, Saghatchian M. Centre René Huguenin, Saint Cloud, France; Bayer Diagnostics, Leverkusen, Germany; Hopital Européen Georges Pompidou, Paris, France

Background: Serum HER-2/neu extracellular domain (sHER-2) is elevated in 20-30% of metastatic breast cancer patients, its variations under trastuzumab treatment have not yet been described.

Materials and Methods: sHER-2 was measured by ELISA (OSDI, Oncogene Science, Tarrytown, NY) in serial blood samples from 24 patients drawn before and during trastuzumab treatment (alone, n=3 or associated to taxanes, n=21). Treatment responses, evaluated from clinical records and CT scans, were grouped into 4 categories : progressive (PD), or stable disease (SD), partial response (PR), important or complete regression (CR). Cumulated trastuzumab dose at each blood sampling were recorded. Non parametric statistics were used for comparisons.

Results: Patients received 2507 ± 1928 mg of trastuzumab in 20.92 ± 15.19 weekly injections (range 5-70). 94 sHER-2 levels were measured and matched to clinical responses. Median sHER-2 was 54.27 ng/ml (range 7.54-2565.00 ng/ml) in samples from PD patients (n=32), 13.56 ng/ml (range 9.48-29.55 ng/ml) for SD patients (n=6), 16.70 ng/ml (range 5.80-212.41 ng/ml) for PR patients (n=30) and 12.94 ng/ml (range 6.60-31.75 ng/ml) for CR patients (n=26). Overall, sHER-2 was found to be highly correlated to clinical treatment responses (P< 0.0001) and to trastuzumab cumulative doses (P 0.0002).

Discussion: Serial assays of sHER-2 before and during trastuzumab treatment are correlated to patient responses and might be a valuable tool to monitor treatments by anti-HER-2/neu monoclonal antibodies.

427 Evaluation of serum HER-2/neu for outcome assessment and monitoring of Herceptin plus combination chemotherapy in metastatic breast cancer.

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Introduction: Monitoring of the shed antigen of HER-2/neu in serum (sHER-2/neu) is useful for the management of metastatic breast cancer patients (pts). We evaluated sHER-2/neu in pts on Herceptin plus combination chemotherapies and correlated the results to the clinical outcome. Material/methods: Weekly samples were collected from 54 pts. The initial sample was obtained prior to initiation of Herceptin-based therapy at the time of disease progression. Restaging was done using standard methods of evaluation. sHER-2/neu was measured using the Bayer Immuno1 and the Oncogene Science HER-2/neu assays with an upper limit of normal of 15 ng/mL. Results: Sixteen of 45 (35.6%) women with a complete clinical and biochemical data set had a response (PR/CR), 14 of 45 (31.1%) achieved SD and 15 of 45 (33.3%) showed PD to Herceptin-based therapy. The mean baseline sHER-2/neu concentration was 284.4 ng/mL (median: 22.4 ng/mL). Probability of response increased with the baseline sHER-2/neu level (23.1% for sHER-2/neu <15 ng/ml; 35.5% for sHER-2/neu of 15-50 ng/mL; 46.7% for sHER-2/neu > 50 ng/mL) but due to moderate pt numbers this association was not significant (p>0.05). An ROC curve for PD vs. CBR (CR+PR+SD) was produced (AUC=0.78, 95% CI: 0.6-0.94) indicating that sHER-2/neu at 3-5 weeks relative to baseline were significant discriminators of response. The ROC curve indicated that at a relative value of 65% of baseline, the sensitivity for the detection of PD was 90%. Using only patients who responded or progressed, a second logistic analysis confirmed this association between relative values of sHER-2/neu at 4 weeks and best response with the AUC increasing to 0.82 (95% CI: 0.66-0.99; sensitivity 92%; specificity 64%). The specificity for monitoring of PD (percentage of pt with a change in sHER-2/neu<15%) was 82.7%. Conclusions: Monitoring of sHER-2/neu is an effective tool to evaluate response to Herceptin plus chemotherapy. If a pt does not show a 65% decrease in comparison to baseline at week 4, she has a 90% probability not to benefit from therapy, warranting thorough and earlier restaging and possibly change of chemotherapy. This time interval is clinically helpfull as most chemotherapy regimens work with q3w administration.

428 Serum HER2 extracellular domain (ECD) correlates with HER2 status by immunohistochemistry (IHC) and fluorescent in-situ hybridization (FISH) in metastatic breast cancer (MBC) patients (pts) treated with weekly (W) trastuzumab (H) and paclitaxel (T).

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Introduction: Elevated HER2 ECD is found in the serum of 20-25% of MBC pts (Leitzel et al, JCO 1992, 10:1436) and it is likely a function of:

1.tumoral HER2 receptor density
2.rate of ECD cleavage
3.tumor burden

We explored ECD correlation with HER2 status By IHC and FISH in a cohort of MBC pts treated with W T+H.

Materials and Methods: 95 pts with HER2 over-expressing and non over-expressing MBC were treated with W T+H. (Seidman et al, JCO 2001 10:2587) We performed a retrospective analysis to evaluate the correlation between pre-treatment (Tx) HER2 ECD and HER2 status by IHC (DAKO HercepTest and CB11) and FISH (VYSIS). Pre-Tx HER2 ECD levels were available from stored serum samples for 47 (49%) pts. HER2 ECD was measured using the Immuno 1 immunoassay for HER2 by Bayer Corporation; the elevated cutoff was defined as >15 ng/mL.

Results: 30 pts (64%) had pre-Tx elevated ECD. Correlation of pre-Tx ECD with HER2 status by IHC and FISH is shown below:

HER2 status	Pre-Tx ECD >15ng/mL	Fisher's Exact
DAKO 3+	92% (11/12)	p<0.01
CB11 2,3+	92% (11/12)	p<0.01
FISH ≥2	82% (14/17)	p=0.01

Conclusion: In our cohort, a statistically significant correlation was observed between HER2 ECD and HER2 status by IHC and FISH. Further investigation of this observation is necessary to evaluate a possible role for serum ECD assessment in selecting pts for H-based Tx.

Supported in part (reagents) by Bayer Corporation, Tarrytown, NY.

429 Patients with MBC prospectively selected by FISH derive clinical benefit from first-line treatment with Herceptin® plus a taxane.

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Background: Reports have shown patient selection based on *HER2* gene amplification by FISH provides improved clinical benefit from Herceptin compared with selection by IHC. A retrospective analysis of the pivotal clinical trials revealed that adding Herceptin to chemotherapy provided a significant survival benefit in patients who were FISH+ (OR, 0.71; 95% CI, 0.54-0.92; *P*=0.009) but not in the FISH- group (OR, 1.11; 95% CI, 0.70-1.80; *P*=NS).

Patients and Methods: A multicenter trial evaluating outcomes in patients prospectively selected by FISH to receive Herceptin for MBC is being conducted throughout the US. Patients are enrolled based on local or central HER2 testing (3+ by IHC or FISH+); local IHC and FISH results are confirmed centrally. Treatment consists of Herceptin (4 mg/kg week 1 followed by 2 mg/kg/week) in combination with taxol or taxotere per physician's discretion. Herceptin is continued until disease progression. Cardiac monitoring by serial LV assessments and physical examinations are performed.

Results: 63 patients have been on study for at least 6 months; response data are available for 57. Of these, 7 (12%) had CR, and 22 (39%) had PR, for a total RR of 51%. Ten (17%) additional patients had SD, for a clinical benefit rate of 68% [CR + PR + SD ≥6 months]. Overall RRs were 61% and 38% in FISH+ and FISH- patients, and clinical benefit rates were 81% and 56%, respectively. Seven patients were FISH+ and IHC <3+; the RR was 57% with a clinical benefit rate of 71%. There was no difference in RR or clinical benefit rate with either taxane regimen. Average LVEF was 63.5% at study entry and 60.1% at 16 weeks (n=33). Although many patients have been on study for <6 months, among all patients enrolled (n=221), there have been 2 reported cases of CTC grade 3 CHF; 1 of the 2 patients had a previous history of viral cardiomyopathy and a baseline LVEF of 32%.

Conclusions: These preliminary data suggest that patients who are prospectively selected by FISH derive an important clinical benefit from Herceptin plus chemotherapy. Although small numbers, these data also suggest that patients who are <3+ by IHC and FISH+ also derive benefit from Herceptin plus chemotherapy. Thus far, there is a low incidence of clinical cardiotoxicity. Data on all enrolled patients will be presented.

430 Cardiac safety of epirubicin/cyclophosphamide (EC) alone and in combination with herceptin in women with metastatic breast cancer (MBC).

Thomssen CH, Eidtmann H, Untch M, Meerpohl HG, du Bois A, Schaller G, Kuhn W, Jackisch CH, Kreienberg R, Wallwiener D, Weise W, Emons G, Langer B, Lueck HJ. Univ Hosp Eppendorf, Hamburg; Univ Hosp, Kiel; Univ Hosp Grosshadern, Munchen; St. Vincentius-Hosp, Karlsruhe; Dr. Horst-Schmidt Hosp, Wiesbaden; Univ Hosp Benjamin Franklin, Berlin; Univ Hosp, Munchen; Univ Hosp, Munster; Univ Hosp, Ulm; Univ Hosp, Tubingen; Univ Hosp, Magdeburg; Univ Hosp, Gottingen, Germany; Hoffmann-La Roche Ltd, Basel, Switzerland; Med Univ Hosp, Hannover, Germany

Introduction: Concomitant use of doxorubicin (A)/cyclophosphamide (C) + Herceptin (H) is associated with increased risk of cardiotoxicity compared to AC alone in MBC. Epirubicin (E) is considered to be less cardiotoxic than A. This study assesses the incidence of cardiotoxicity with first-line EC+H and EC alone in women with MBC.

Design: Patients with HER2+ MBC received EC+H. A control group with HER2- MBC received EC alone. For the first 26 HER2+ patients, E dose was 60 mg/m^2 (EC60+H). LVEF was evaluated prospectively and independently by echocardiography. Incidence of dose-limiting cardiotoxicity (DLC) after 6 cycles of EC60+H determined whether E dose would be escalated to 90mg/m^2. No DLC was observed with 6 cycles of EC60+H; E dose was escalated in the subsequent 25 patients. To date, 24 women with HER2- MBC have received EC90 alone.

Results: 21 of 46 evaluable patients treated with EC+H showed a measurable decrease of LVEF (>10%); one symptomatic cardiac event was observed during treatment with EC+H. Three patients subsequently developed symptomatic congestive heart failure (one with EC60+H and two with EC90+H) during treatment with H alone.

	EC60+H	**EC90+H**	**EC90**
Patients recruited/evaluated	26/24	25/22	24/14
Baseline LVEF, % (range)	70 (57-82)	72 (62-90)	69 (58-79)
LVEF decrease >10% (n)	8/24	10/22	3/14
LVEF decrease >10% to absolute value <50% (n)	1*/24	2*/22	0/14
Response rate, %	62	71	13

*These events occurred >5 months after EC therapy was completed.

Conclusions: Compared to the incidence of cardiac events with AC+H (NYHA class III/IV: 19%, Slamon et al. 1998), the safety of EC+H appears promising while efficacy is similar. The decision whether to continue the trial by treating a further 75 patients with EC+H and the dose of E will be reported.

431 Phase I trial of Herceptin (H) and navelbine (Nvb) for patients with HER-2/*neu* (+) advanced breast cancer.

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Navelbine (vinorelbine), a 3rd generation vinca, has significant activity in breast cancer. Recent preclinical and clinical data have suggested a synergistic interaction between H and several chemotherapeutic agents, and particularly promising findings have been noted with H and Nvb. The precise mechanism of interaction between HER-2/*neu* targeted therapies and cytotoxics is unclear. Recently it has been shown that vincas may affect several members of the MAP kinase pathway, this likely plays an important role in induction of apoptosis by these agents. Further, it appears that signal transduction pathways downstream from the HER receptor family may converge with these former pathways. Elaboration of these findings will provide a mechanistic explanation of this synergy. While many trials of Nvb have utilized weekly therapy, substantial dose reductions or delays occur frequently, particularly on day 15. Thus, we have designed a phase I trial to evaluate escalating doses of Nvb, on a day 1 and 8 schedule q 21 days, in combination with H. The primary objective of this trial is to determine the MTD (maximally tolerated dose) of Nvb, utilizing a day 1 and 8 schedule, with conventionally dosed H (4 mg/kg load > 2 mg/kg weekly). Secondary objectives include evaluating: 1) the effect of Nvb and H on expression or activation of several members of the MAP kinase pathway(s), including JNK, Akt, and ERK; and 2) clinical efficacy. Eligibility criteria include: locally advanced breast cancer refractory to 1st line chemotherapy or metastatic breast cancer; 1-3(+) overexpression of HER-2/neu (immunohistochemical analysis); adequate hepatic, renal, hematopoietic, and cardiac function; and written informed consent. 13 patients have been enrolled and treated. The median age is 58 years old (range 44-77). Successive cohorts have been treated with Nvb at 20, 25, and 30 mg/m2, on a day 1 & 8 schedule q 3 weeks. Toxicities attributable to therapy are primarily hematologic, and include grade 3/4 neutropenia at 30 mg/m2 (3/6 patients in all cycles). The final cohort, per protocol, will be expanded. Final toxicity, laboratory, and clinical data will be available for presentation.

432 Monitoring serum HER-2/neu levels in metastatic breast cancer patients undergoing Herceptin® therapy regimens.

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Background: The HER-2/neu Oncoprotein is an important therapeutic target for treating women with metastatic breast cancer (MBC). Assessing HER-2/neu status during MBC can be an important tool in the treatment and management of women with MBC. The Oncogene Science/Bayer HER-2/neu Microtiter ELISA measures serum levels of HER-2/neu extracellular domain (ECD), allowing continuous assessment of HER-2/neu status in metastatic patients during therapy. The anti-HER-2/neu antibody Herceptin® (Trastuzumab) has become a more widely used therapy and was recently shown to inhibit basal and activated HER2 ECD cleavage in breast cancer cells (Molina et al.).

Materials and Methods: A retrospective study was performed to evaluate the ability of the ELISA to accurately monitor the serial changes in serum HER-2/neu ECD levels in 57 women with MBC treated over a 2 year period with Herceptin® combined with chemotherapy. The ECD levels were quantitated in pretreatment and in subsequent serial samples from these women. Previous studies have shown no interference of Herceptin® in the ELISA.

Results: Serial changes in serum HER-2/neu ECD levels reflected the clinical course of disease. Women with progressive breast cancer while on Herceptin®/Chemotherapy had serially increasing ECD levels, while women responding to a Herceptin®/Chemotherapy treatment regimen had serially decreasing HER-2/neu ECD levels. Of special note was that 39 of the 57 MBC patients (68%) had at least 2 samples that were greater than the normal cutoff of 15ng/ml, similar to results observed previously with patients on other treatment regimens. Our studies suggest that over 50% of women with Metastatic disease have HER-2/neu positive tumors according to serum HER-2/neu levels, which is in contrast to tissue studies with the primary tumors demonstrating that only 20-30% of women with Primary Breast Cancer have HER-2/neu positive tumors.

Conclusion: Our studies demonstrate that monitoring serum HER-2/neu ECD levels during MBC may be an important test for managing the treatment of women with HER-2/neu positive tumors, including those on Herceptin®.

433 Monitoring clinical response to trastuzumab (Herceptin) therapy based on measurements of serum HER-2/neu in metastatic breast cancer.

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Background: Amplification and over-expression of HER-2/neu is a predictor of poor prognosis in breast cancer patients. Patients who are HER-2/neu tissue positive are eligible for Herceptin therapy at recurrence of disease. In this study we evaluated the role of serum HER-2/neu in monitoring patients on Herceptin based therapy.

Materials and Method: A total of 37 patients who were tissue positive (IHC 3+ or 2+ using Dako's Herceptest) at diagnosis were treated with Herceptin at recurrence. A cutoff of 15 ng/ml was derived based upon an analysis of serum HER-2/neu levels in the sera of 242 healthy women. Normal longitudinal variability was determined to be 15% by analyzing 6 serial samples from 38 normal healthy females. Serum samples were drawn at baseline before the initiation of drug therapy and at various intervals during therapy. Serum HER-2/neu was measured on the Bayer Immuno 1 system. Objective clinical assessments were recorded according to the WHO criteria.

Results: A total of 291 serial samples from 37 patients were evaluated. For each pair of serial measurements (visit to visit), an increase of equal to or greater than 15% was considered to indicate progression, and a change of less than 15% was considered to indicate lack of progression.

The overall concordance of serum HER-2/neu levels with clinical status in patients on Herceptin therapy was 76.1% (CI 74.4% - 81.1%) with a Predictive Value of 87.1% (CI 82.7% - 91.5%) for drug response.

Conclusion: The data shows the utility of serum HER-2/neu in monitoring clinical response of patients on Herceptin based therapy.

434 Multinational phase II study of navelbine (N) and herceptin (H) as first-line therapy for HER2-overexpressing metastatic breast cancer (HER2+ MBC).

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Navelbine and Herceptin combination therapy has demonstrated preclinical synergy and documented clinical benefit for HER2+ MBC as second-line therapy (Burstein et al.; J Clin Oncol 2001; 19; 2722-30). Based on these encouraging results, we developed an international trial to assess the efficacy and safety of N and H as first-line therapy. Methods: Eligible patients (pts) have HER2+ (either FISH + or IHC 3+) MBC, measurable disease, KPS >70%, normal baseline LVEF and no prior H or chemotherapy for MBC. Patients were treated with weekly iv doses of N (30 mg/m^2) and H (4 mg/kg loading dose, then 2 mg/kg) within 4-week cycles. Patients were restaged every 8 weeks. Results: From 10/00 to 02/02, 41 of the planned 60 pts were enrolled onto the study. Patient characteristics are: median age, 51 (range 30-71) yrs; prior adjuvant/neoadjuvant chemotherapy, 66% (anthra 32%, taxanes 12%, both 7%); prior hormonal therapy, 51%; visceral metastasis 60%. Currently 33 pts are evaluable for response having received at least 2 cycles. The objective clinical response is 70% (six CRs and 17 PRs); 7 pts have stable disease and 3 pts progressed. 39 pts are evaluable for toxicity. 170 cycles have been administered (median 4, range 1-10); the weekly median administered dose was 20 mg/m^2 for N and 2 mg/kg for H. WHO grade 3/4 neutropenia occurred in 46% of cycles while infection was observed in 2% of pts. Non-haematological grade 3/4 toxicity: asthenia was noted in 3 pts, 1 pt came off study for Grade 3 cardiac toxicity with decline in LVEF to 38% and symptomatic cardiac dysfunction that resolved with therapy and 1 pt experienced grade 3 constipation. No severe neuropathy, nausea, vomiting or alopecia have been reported. Conclusion: These data from a multinational trial confirm that N+H is very active and well-tolerated as first-line therapy for HER2+ MBC. Final results will be presented.

435 Weekly trastuzumab (T) and paclitaxel (P) in metastatic breast cancer (MBC). The impact of taxane free interval (TFI) on treatment outcomes.

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T is known as an active agent in HER2/neu overexpressing MBC. In the prospective study we assessed efficacy, safety and toxicity of T and P in patients progressing despite previous therapy. Patients and methods: We accrued 17 patients with histologically confirmed MBC, Karnofsky PS ≥ 60%, median age 50[36-66], pretreated with at least two regimens. HER2/neu was tested by Herceptest(DAKO). Fifteen specimens were 3+ and two 2+. All patients except 1 were pretreated with taxanes. TFI was defined as a time from last taxane administration until the beginning of the study for every enrolled patient. TFI > 1 year was found in 7 patients and TFI < 1 year in 9 patients. T was given 4mg/kg i.v. followed by 2mg/kg i.v. weekly. P was given 80mg/m2 i.v. weekly. Results: In the intent to treat population we found objective response (OR) in 10 patients (59%), including 2 complete responses (CR). In the subset with TFI > 1 year 4 ORs were observed including 1 CR (RR 57%) and in the subgroup with TFI < 1 year 6 ORs with 1 CR (RR 67 %). TFI was not significant for OR (p<0,4349). Median time to progression (TTP) was 6 month. Patients with TFI > 1 year tend to have longer TTP (p=0,0201). Median OS has not been reached thus far. We administered 599 cycles including 473 cycles of T and P with no dose adjustment. One patient developed allergic reaction on the first T infusion.The most common toxicity was T infusion related pyretic reaction observed in 6 patients. Ejection fraction decline grade (G) 2 occurred in 1 patient and G3 also in 1 patient. Six patients experienced G3 neuropathy. There were observed 1 episode of G4 neutropenia and G3 anemia. We noted 4 episodes of G3 infection without neutropenia. G3 elevation of liver function tests occurred in 6 patients with no need for dose reduction. There were observed 1 episode of G3 hyperglycemia and 1 episode of G3 weight gain. Other G3 or 4 toxicity was not detected. Conclusion: T and P have shown activity and good tolerability in HER2/neu overexpressing MBC. Tumor response in 10 taxanes pretreated patients was independent on TFI, but patients with longer TFI tend to remain longer progression free.

436 Chemotherapy with trastuzumab plus vinorelbine in patients with erb-B2 overexpressed tumor is active in metastatic breast cancer.

Filipovich E, Mayordomo JI, Isla D, Andres R, Escudero P, Alvarez I, Saenz A, Polo E, Lara R, Tres A. Universitary Hospital, Zaragoza, Spain

Introduction: Trastuzumab is an anti-erbB2 humanized monoclonal antibody with proven activity in patients with erb-B2 overexpressing metastasic breast cancer (MBC). Following description of enhanced antitumor activity when trastuzumab is combined with paclitaxel, a comprehensive search of cytotoxic drugs active in MBC that are synergistic with trastuzumab is ongoing. Vinorelbine is one of the drugs with the best combination index. Objective: We have assessed the activity of vinorelbine plus trastuzumab in erb-B2 overexpressing MBC. Patients and methods: From January 1999 to August 2001, 11 patients have been treated with vinorelbine 25mg/m2 / week plus trastuzumab 4mg/kg (first week) followed by 2 mg/kg/week. Results: Median age was 43 (range 31-67). Number of prior chemotherapies for MBC was 2 (range 0-3). Median time from diagnosis to first relapse was 15 months (range 10-48). All patients had +++ erb-B2 by inmunohistochemistry in the primary tumor. Seven patients (63%) had liver metastases. Overall, 151 treatment courses were given (median=11 courses; range=1-35). The most relevant toxicity was grade 3 leukopenia requiring omission of vinorebine in 17 courses. All 9 patients receiving more than 10 weeks of treatment developed grade 1-2 peripheral neuropathy, that was treated with gabapentin and did not require treatment discontinuation. One patient achieved a complete response, 7 patients had a partial response, 2 had stable disease for more than 2 months and 1 had progressive disease. Overall response rate was 72%. Median time to progression was 7 months (range 0-18+). Median survival was 9 months (range 1-18+). Conclusions: Trastuzumab plus vinorelbine is an active and well tolerated treatment option for patients with erb-B2 overexpressing metastasic breast cancer.

437 Phase II study of gemcitabine, paclitaxel and trastuzumab in metastatic breast cancer; a Hoosier Oncology Group trial.

Miller KD, Sisk J, Gize G, Nattam S, Ansari R, Vinson J, Sledge G. Indiana University, Indinapolis, IN; Consultants in Blood Disorders and Cancer, Louisville, KY; Ft. Wayne Hematology and Oncology, Ft. Wayne, IN; Michiana Hematology-Oncology, South Bend, IN; Walther Cancer Institute, Indianapolis, IN

Background: The addition of trastuzumab to paclitaxel dramatically improves response rate and survival. Both paclitaxel and gemcitabine are active agents in the treatment of breast cancer and can be given in combination at full dose. We hypothesized that the triplet combination of gemcitabine, paclitaxel and trastuzumab may further improve these results.

Patients and Methods: Patients with newly diagnosed Her-2 overexpressing (gene amplification by FISH or 2-3+ protein expression by IHC) metastatic breast cancer were treated with paclitaxel 175 mg/m2 over 3 hours on day 1 plus gemcitabine 1200 mg/m2 on days 1 and 8 plus trastuzumab 4 mg/kg loading dose on day 1 followed by 2 mg/kg weekly. Treatment cycles were repeated every 21 days for a maximum of 6 cycles; responding or stable patients continued single-agent trastuzumab weekly until disease progression.

Results: From 9/99 to 8/01, 45 patients were enrolled. Median age was 54 (range 32-78); median KPS 90; visceral disease predominated in over half of patients. Treatment was generally well tolerated; 40% of patients developed grade 4 neutropenia but infectious complications were rare. Grade 4 anemia and thrombocytopenia occurred in 1 and 3 patients respectively. 3 patients developed CHF though only one had a > 15% decline in LVEF. 5 patients developed ≥grade 3 pulmonary toxicity. Objective response rate was 62% (4 CR; 24 PR); median time to progression was 196 days. Median survival has not been reached.

Conclusions: Combination treatment with gemcitabine, paclitaxel and trastuzumab is well tolerated and highly active. Randomized trials are needed to further evaluate this regimen.

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439 Final results of the Minnie Pearl Cancer Research Network first-line trial of weekly paclitaxel/carboplatin/trastuzumab in metastatic breast cancer.

Yardley DA, Greco FA, Hainsworth JD, Houston GA, Broome CM, Bramham J, Patton JF, Thompson DS, Erland JB, White MB, Burris III HA. Sarah Cannon Cancer Center/Tennessee Oncology, PLLC, Nashville, TN; Jackson Oncology Associates, Jackson, MS; Northern Virginia Oncology Group, PC, Fairfax, VA; Graves-Gilbert Clinic, Bowling Green, KY

A phase II multicenter pilot study of weekly paclitaxel, carboplatin, and trastuzumab was initiated in October 1999 as first-line treatment in HER2 overexpressing metastatic breast cancer. 61 patients (pts) with 2+ or 3+ HER2 expression by immunohistochemistry (IHC) were enrolled. Pt demographics: median age 52 years, ECOG 0-1, 50% ER+, > 50% with hepatic involvement, and prior adjuvant therapy in 33 pts. 20 pts were newly diagnosed with stage IV disease at presentation. After single agent induction trastuzumab, weekly paclitaxel 70 mg/m2, carboplatin AUC 2, and trastuzumab 2 mg/kg was administered 6 consecutive weeks followed by 2 weeks rest. Trastuzumab was discontinued in pts demonstrating PD to induction therapy. 779 weekly treatments have been administered. Only 33% grade 3/4 leukopenia was noted with no febrile neutropenia. Grade 3/4 non-hematologic toxicity was rare with 7% fatigue, 4% diarrhea, and 4% neuropathy. 4 pts experienced asymptomatic LVEF declines with subsequent recovery documented. Retrospective FISH HER2 analysis was obtained for 49 pts to date. Results: ORR for all pts was 66% with a median overall survival of 29.3 mo and a median TTP of 12 mo. FISH + pts demonstrated an 89% ORR with median TTP of 19 months and a median survival having not yet been reached at 30 months of follow-up. In FISH - pts, a 44% ORR, median TTP of 8.5 mo, and median survival of 19 months was noted. Conclusion: Weekly paclitaxel, carboplatin, and trastuzumab is well tolerated and highly active in HER2 overexpressing metastatic breast cancer. These results confirm the activity of the q21 day taxane/platinum/trastuzumab combination reported by Slamon and Nabholtz. HER2 amplification by FISH better selects those patients who may derive the most clinical benefit from weekly paclitaxel, carboplatin and trastuzumab as compared to selection by IHC (2+/3+).

440 Efficacy and safety of Herceptin in women with Her2-positive (HER2+) metastatic breast cancer (MBC) who have progressed on a prior herceptin-containing regimen.

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Introduction: Data from clinical trials support the use of Herceptin until disease progression in women with HER2+ MBC, whether it is used alone or in combination with chemotherapy. It is not known whether additional benefit is obtained by continuing treatment beyond progression. Therefore, we retrospectively evaluated patients treated with Herceptin beyond progression.

Methods: Charts of women with HER2+ MBC who had received ≥2 Herceptin regimens were reviewed retrospectively.

Results: Data for 105 women were collected. Tumour HER2 status was: IHC 3+ 75; IHC 2+ 13; IHC positive (not specified) 3; IHC negative 3; and FISH positive 22 (patients tested using IHC and FISH: 11). Median overall survival was 29 (95%CI 22.7-45.0) months. 77 of 105 women received Herceptin monotherapy (n=27) or in combination with taxane (50) as initial Herceptin regimen; the most common second regimens were Herceptin plus vinorelbine (33) or taxane (21) or monotherapy (11). Response rates and TTP are shown in the Table. Response to the first Herceptin regimen did not preclude response to the second; patients also responded to a second regimen having failed to respond to the first.

	Response rate, %(CR, PR)		TTP, weeks (range)	
Regimen	First regimen	Second regimen	First regimen	Second regimen
Herceptin monotherapy	41 (1,10)	36.4 (1,3)	23 (3-73)	30.5 (18-68)
Herceptin + taxane	38 (5,14)	38.0 (0,8)	24 (0-79)	24 (3-72)
Herceptin + vinorelbine	-	27.3 (0,9)	-	26 (3-108)

24 cardiac events were reported in 22 patients. The majority received further Herceptin regimens after experiencing an event. No fatal cardiac events were reported. Of 13 patients with baseline cardiac risk factors, only 3 experienced cardiac events.

Conclusions: In this heterogeneously treated population, median overall survival, response rates and TTP with the first and second regimen compare favourably with those observed in clinical trials. The incidence of cardiac events was no greater than expected. Continuing Herceptin beyond disease progression appears to be feasible and warrants further study.

441 Phase II study of neoadjuvant trastuzumab plus docetaxel for locally advanced and metastatic breast cancer that overexpresses HER2/neu: a preliminary report.

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Background: Trastuzumab is a selective humanized monoclonal antibody to the HER2-receptor. Trastuzumab/chemotherapy combinations have already shown superior results in metastatic breast cancer patients. The purpose of this study is to determine the clinical efficacy of the combination of neoadjuvant trastuzumab and docetaxel. **Methods:** Women with HER2-overexpressing locally advanced or metastatic breast cancers, normal liver and kidney function, and adequate performance status were included. Patients received IV trastuzumab, 4 mg/kg loading dose, then 2 mg/kg weekly. On day 22, docetaxel 100 mg/m^2 every 3 weeks for 4 cycles was added to weekly trastuzumab. Patients then underwent surgery and subsequent 4 cycles of AC (60/600) without trastuzumab. Weekly trastuzumab was resumed 1 month after completion of AC and continued for a year. LVEF by serial MUGA scans was monitored throughout the study. **Results:** Preliminary results from 16 patients with median follow-up of 11 months (range 3-27) are reported here. Of these, 7 patients (43.8%) had inflammatory breast cancer, and 5 patients (31.3%) had stage IV breast cancer (metastases to lung, liver and bone) at the time of diagnosis. Thirteen of 16 patients (81.3%) had objective clinical response, with a clinical complete response in 7 patients (43.8%). All 7 patients with inflammatory breast cancer demonstrated clinical response, with 5 of 7 (71.4%) complete responders. Two patients (12.5%) had reversible decline in cardiac function. Six patients have relapsed, one with local skin recurrence (6.3%), and five with distant recurrence of whom one had liver metastasis (6.3%), and four had brain metastases (25%). **Conclusion:** Combined neoadjuvant trastuzumab and docetaxel induced high clinical response rates for HER2-overexpressing breast cancer, particularly for inflammatory breast cancer. A high rate of brain metastasis was noted, particularly in patients with baseline metastatic disease. Additional patients are needed to confirm the data.

442 Brain metastases during Herceptin therapy: improved survival compared to patients not treated with Herceptin.

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We have previously reported that metastatic breast cancer patients receiving Herceptin are at increased risk for the development of brain metastasis. The aims of this study were to compare the survival of breast cancer patients (pts) with brain metastasis who received Herceptin to those pts who do not receive Herceptin and to determine the impact of brain metastasis on the overall survival of pts receiving Herceptin. Two groups of pts were studied: one group was receiving Herceptin for the treatment of HER-2/neu overexpressed breast cancer and another group without HER-2/neu overexpressed tumors never received Herceptin. Patients diagnosed with brain metastasis prior to receiving Herceptin were excluded from study. Available clinical data included patient age, tumor characteristics, dates of breast cancer diagnosis, brain metastasis, dates of Herceptin institution and discontinuation, and date of death. A total of 103 pts receiving Herceptin was identified with 18 pts diagnosed with brain metastasis prior to Herceptin treatment excluded from subsequent analysis. Twenty-two of the remaining 85 Herceptin pts (26%) subsequently developed brain metastasis. A comparison group of 60 metastatic breast cancer pts with brain metastasis was studied. The median survival from the time of diagnosis of brain metastasis of the 22 Herceptin pts with brain metastasis was 258 days compared to 92 days for the 60 non-Herceptin pts with brain metastasis (Chi square = 6.35, $p<0.02$). We also compared the survival of pts from the institution of Herceptin. Herceptin pts without brain metastasis (63 pts) had longer survival (median survival 800 days) compared to the Herceptin treated pts who developed brain metastasis (median survival 280 days from starting therapy) and this difference was significant (Chi square = 5.69, $p<0.02$). We found that brain metastasis was associated with a worse prognosis. For pts treated with Herceptin, the development of brain metastasis was associated with a worse prognosis than those without brain metastasis. However, pts receiving Herceptin who developed brain metastasis had a better prognosis than those who developed brain metastasis without Herceptin.

443 Multicenter randomized phase II study of docetaxel (Doc) given q3w vs q1w plus trastuzumab (Tra) as first line therapy for HER2 overexpressing adjuvant anthracyclin pretreated metastatic breast cancer (MBC).

Raab G, Brugger W, Harbeck N, Heidemann E, Schaller G, Wallwiener D, Bacchus L, De Wit M, Eidtmann H, Friese K, Goebel R, Hilfrich J, Hoeffken K, Kreienberg R, Kuhn W, Loehr A, Nitz U, Wolf H, Buechele T, Eiermann W. Frauenklinik vom Roten Kreuz, Muenchen; Klinikum, Villingen-Schwenningen; Klinikum Rechts der Isar, Munich; Diakonissen Krankenhaus, Stuttgart; University-Hospital Benjamin Franklin, Berlin; University, Tuebingen; Robert-Bosch-Krankenhaus, Stuttgart; University Eppendorf, Hamburg; University, Kiel; University, Rostock; Evang. Krankenhaus, Oberhausen; Krankenhaus der Henriettenstiftung, Hannover; University, Jena; University, Ulm; University, Bonn; Dr. Horst-Schmidt-Kliniken, Wiesbaden; University, Duesseldorf; Kreiskrankenhaus, Leonberg; Aventis Pharma Germany, Bad Soden, Germany

Introduction: Based on preclinical data suggesting synergy between Tra and Doc and on clinical experience with this combination in phase I/II trials with different schedules, we performed a randomised phase II trial to characterize the efficacy and cardiac safety of this combination with Doc given either weekly or q3w in anthracyline (A) pretreated patients (P).

P and Methods: Eligible P included HER2+ (either FISH+ or IHC 3+) MBC, measurable disease, PS 0-2, normal baseline LVEF. Treatment was Tra (4 mg/kg first dose, 2 mg/kg q1w thereafter until progression) and Doc either 100 mg/m^2/ q3w (max 7 cycles) Arm A or 35 mg/m^2 weekly x 6 qw8 (max 3 cycles) Arm B). LVEF was reassessed every 8 weeks. Due to the approval of Tra in 2000, resulting in a widespread use outside of clinical trials, the accrual goal of 50 P per arm could not be met and the study was closed with an enrollment of 25 P (13 Arm A, 12 Arm B) from 18 centers between 01-00 and 08-01.

Results: Safety (Arm A n=13/Arm B n=12): Grade (G) 3/4 neutropenia 92%/0%; febrile neutropenia 23%/0%. Cardiac toxicity(cumulative A pretreatment median 600mg): G 2 8%/0%, G 1 23%/25%. No clinically relevant G 3/4 non hematologic toxicities. Efficacy (n=24): ORR 63% (CR/PR n=4/11), SD n=7, median TTP 8.3 months.

Conclusions: This trial confirms the feasibility and efficacy of the Tra/Doc combination. Both arms could be delivered safely without clinically relevant cardiac toxicities after anthracycline pretreatment. The hematologic toxicity profile favored the weekly schedule.

This study was supported by Aventis Pharma Germany and Hoffmann-La Roche Germany.

444 Clinical efficacy & toxicity of Herceptin in metastatic breast cancer patients with tumours that overexpress Her-2 neu: the Australian expanded access program.

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Introduction An expanded access program of Herceptin (H) in Australia, was commenced in December 1998.

Patients and treatment 46 women (median age 52y, range 32 - 80y) with metastatic breast cancer were treated. Her-2 testing by IHC was performed in 45 patients (pts). Tumours were assessed as 3+, 2+ or positive unspecified in 54%, 20% and 24% patients, respectively. The majority of patients (52%) received H as ≥4th line of therapy.

Results 48% of pts received H for ≥13 weeks . Clinical benefit defined as objective response (42%) or stable disease (20%) was achieved in 62%. H in combination with a Taxane or Vinorelbine resulted in a clinical benefit (89%) more frequently than H alone (44%). Clinical benefit was achieved in 50% of pts receiving H as 2nd, 62% of 3rd and 65% of those receiving 4th line therapy. Severe side effects were uncommon: headache 7% and fatigue 4% being the most common severe complaint. 21% of 19 pts with fatigue, 64% of 14 pts with pyrexia and 18% of 40 pts with any gastrointestinal side effect were thought to be due to H. 17 pts progressed with a median TTP 259 days (95% CI 207-370 days). Median TTP for pts receiving H as 2nd, 3rd or 4th line therapy was 207, 370 and 328 days, respectively. Median TTP was 259 days for H as monotherapy, compared to 328 days for those receiving combination therapy.

Conclusion H produced clinical benefit in almost two-thirds of patients despite 80% of patients having received at least 2 previous lines of chemotherapy. Severe side effects were infrequent. H used in combination with chemotherapy resulted in a greater proportion of patients experiencing clinical benefit and was associated with longer TTP compared with H monotherapy. Data presented from an Australian population confirms the efficacy and tolerability of H in heavily pretreated patients.

445 Phase II multicenter study to evaluate the efficacy and safety of Tarceva™ (erlotinib, OSI-774) in women with previously treated locally advanced or metastatic breast cancer.

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Tarceva™ is an orally administered selective inhibitor of the epidermal growth factor receptor tyrosine kinase. The efficacy and safety of single-agent Tarceva™ at a starting dose of 150 mg/day was studied in two cohorts of patients with locally advanced or metastatic breast cancer. Patients in cohort 1 had disease progression on or after prior therapy with an anthracycline, a taxane, and capecitabine. Patients in cohort 2 had disease progression on or after at least one prior regimen for locally advanced or metastatic disease. One objective response in the first 21 patients was required to add additional patients to a cohort. Forty-seven patients were accrued to cohort 1 and 22 patients were accrued to cohort 2. Patients in both cohorts had extensive prior treatment. The mean number of prior treatment regimens (hormonal therapy, chemotherapy, or other systemic therapy) was 8 in cohort 1 and 6 in cohort 2. Approximately 40% of patients in both cohorts had previously received trastuzumab. In cohort 1 there was one partial response of 23 weeks duration for an objective response rate of 2.1% (95% C.I. 0%, 11%). Two additional patients had stable disease for 12 and 16 weeks. In cohort 2 no objective responses were observed but one patient has stable disease of >28 weeks duration. The most common adverse events related or potentially related to Tarceva™ included an acneiform or other rash (78%, Grade 3 in 2%), diarrhea (59%, Grade 3 in 4%), asthenia (22%, Grade 3 in 3%), nausea (21%, Grade 3 in 2%), vomiting (13%, Grade 3 in 2%), dry eyes (16%) and anorexia (15%). Evaluation of tumor EGFR status is ongoing. Conclusion: Tarceva™ was well tolerated but had minimal activity in a heavily pretreated population of patients with locally advanced or metastatic breast cancer, including a large number of patients with HER2+ tumors. Studies of Tarceva™ in less heavily pre-treated patients and in combination with other systemic therapies are underway.

446 Phase II trial of the anti-VEGF antibody bevacizumab in combination with vinorelbine for refractory advanced breast cancer.

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Preclinical models suggest that combination therapy with angiogenesis inhibitors and cytotoxic chemotherapy provide superior tumor control compared to single modality therapy, alone. Bevacizumab is a humanized monoclonal antibody that neutralizes VEGF, a mediator of angiogenesis. Side effects reported for use of bevacizumab in early clinical trials include thrombosis, bleeding, hypertension, and proteinuria. We are conducting a phase II trial of bevacizumab in combination with vinorelbine. This regimen was chosen because of laboratory data suggesting efficacy of vinca agents in association with angiogenesis inhibitors, and because of the side effect profile of vinorelbine. Key eligibility criteria include: metastatic breast cancer with measurable disease (RECIST); PS 0-2; prior chemotherapy with one or two regimens for MBC (including trastuzumab if tumor is HER2+), or disease progression within one year of adjuvant chemotherapy; no prior vinorelbine or angiogenesis inhibitor therapy. Patients with bleeding/clotting diatheses, recent surgery, CNS metastases, or proteinuria, and those requiring systemic anticoagulation, were excluded. Patients receive bevacizumab 10 mg/kg every two weeks, and vinorelbine 25 mg/m2 weekly (adjusted for ANC) until disease progression or undue toxicity. Restaging is done every 8 weeks. The principal endpoint is response rate (RECIST), with secondary endpoints of time to progression and safety analyses. Correlative studies with measures of inflammation and angiogenesis are incorporated. The study has a two-stage design, with interim efficacy and safety analyses planned after accrual of 19 patients. Accrual would terminate for fewer than 6 responses among the first 19 patients. More than 6 responses were observed, and accrual currently stands at 43 patients of a projected 56. Toxicity analyses disclosed minor degrees of hypertension, proteinuria, and epistaxis, and one instance of a pericardial effusion. No major bleeding events and no thrombotic events have been noted to date. Other side effects are consistent with historic experience using vinorelbine. Complete toxicity and efficacy data will be presented.

447 A phase I/II pharmacokinetic and dose escalation study of H11 scFv (a human monoclonal antibody fragment).

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Purpose: H11 is a fully humanized, single chain Fv recombinant monoclonal antibody fragment which recognizes a cell surface antigen with multicarcinomic distribution and negligible normal tissue expression. We conducted a phase I/II study with H11 to determine the maximum tolerated dose (MTD), safety, pharmacokinetics and efficacy in patients with various cancers receiving an IV infusion of the drug five times per week for four consecutive weeks. Patients were also evaluated for tumor response using radiological or tumor markers when applicable.

Patients and Methods: The clinical trial was an open label, non-radomized, single center dose escalation study. Patients received a total of 20 doses (five doses per week). Patients enrolled in this study had different solid tumor types, (breast 2; ovary 8; and

colorectal 2) which were metastatic and refractory to standard therapy. All patients had a life expectancy of at least 12 weeks. Patients with CNS metastases and hematological malignancies were excluded.

Results: Twelve patients were enrolled into the study. Safety and efficacy were evaluated at 50, 200 and 400 mg/m(sup)2(/sup). The most common side effects were nausea (42%), dizziness (25%), fatigue (25%), sinus tachycardia (25%), vomiting (25%), and muscle cramps (17%). Two of the six patients experienced anaphylatic reaction at the 400 mg/m(sup)2(/sup) dose. One required systemic corticosteroids and the other did not require any treatment but their infusion rate was decreased. There was no consistent pattern of toxicity observed between the different dose groups. One patient with metastatic breast cancer who showed stable disease after 1 cycle, eventually received 4 additional courses and experienced a partial response in her subcutaneous disease and stabilization in her bony disease. Two patients with ovarian cancer demonstrated stable disease and received a second course of therapy before progressing. A third ovarian cancer patient with stable disease at the end of the first course of therapy elected to discontinue treatment. All remaining patients had progressive disease after one course of H11 therapy. Pharmacokinetic analysis showed that H11 scFv has no appreciable accumulation over time and has a rapid elimination with a half-life of 2.7 hours and 2 hours for the 200 mg/m(sup)2(/sup) and 400 mg/m(sup)2(/sup) dose levels respectively.

Conclusion: These results suggest that H11 scFv was well tolerated at the 50 mg/m(sup)2(/sup) and 200 mg/m(sup)2(/sup) dose. The 400 mg/m(sup)2(/sup) dose was identified as the maximum tolerated dose. Further exploratory efficacy studies are warranted at the 200-400mg/m(sup)2(/sup) dose range.

448 The effect of margins on ipsilateral breast tumour recurrence after breast conservation therapy for node-negative breast cancer.

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Background: Breast conservative surgery (CS) with radiation therapy (RT) is the most commonly utilized treatment for early stage breast cancer in many centres. However, there is controversy about the importance of the pathologic margin status on the risk of ipsilateral breast tumour recurrence (IBTR). This paper examines the effect of the pathologic margin status on IBTR rates in a cohort of women with node-negative breast cancer treated with CS and RT.
Methods: Between August 1980 and December 1994, 452 women with pathologically node-negative breast cancer were treated with CS and RT at Westmead Hospital. Central pathology review was performed in all cases. The final margins were negative for 352 women (77.9%), positive (invasive and/or in situ) for 42 women (9.3%), and indeterminate for 58 women (12.8%). After gross total excision of the tumour, all women were given whole breast irradiation (usually 45-50.4 Gy) and most were given a local tumour bed boost (8-30 Gy).
Results: After a median follow-up of 80 months, 34 women (7.5%) developed an IBTR. The crude 5-year rates of IBTR for women with negative margins, positive margins, and indeterminate margins were 3.1%, 11.9%, and 6.9% respectively. For women with negative margins, the 5- and 10-year actuarial rates of freedom from IBTR were 96% and 92% compared to 88% and 75% for women with positive margins (P=0.003). On univariate analysis, the only factors associated with a significantly higher risk of IBTR were age at diagnosis (P≤0.050) and margin status (P≤0.007).
Conclusion: Our results are comparable to other published reports and they support the association of higher IBTR rates with positive or indeterminate, over negative, pathologic margins. Furthermore, young age (less than 35 years at diagnosis) was found to be associated with the highest risk of IBTR regardless of margin status.

449 Predictors of re-excision findings and recurrence with breast-conserving therapy.

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Purpose: To identify predictors of re-excision findings, local, and distant recurrence in the setting of breast-conserving therapy with radiation.

Methods: The records of 535 patients who underwent breast-conserving surgery followed by radiation for Stage I or II cancer during the years from 1972-1996 were reviewed. Various clinical and pathologic prognostic factors were examined for significance with regard to re-excision findings and recurrence rates using Cox multivariate analysis. Pathologic margin status was classified as negative, close (< =2mm), positive, or indeterminate.

Results: The pathologic margin status was the most important predictor of local recurrence. The freedom from local relapse (FFLR) at six years was 97% for patients with negative pathologic margins and 86% for all others($p < .0001$). There was no significant difference in recurrence rates among patients with close, positive, or indeterminate margins. Analysis limited to patients with negative surgical margins revealed extensive intraductal component , EIC, ($p = .02$) and young patient age ($p = .08$) to be of borderline significance. The presence or absence of residual disease at re-excision did not predict recurrence as long as the final margins were negative. Among patient who underwent re-excision prior to radiation, EIC ($p = .0001$) and young patient age ($p = .03$) were predictive of residual disease in the specimen. Patients with initially close margins and no EIC had a low risk of residual disease at the time of re-excision, as did patients over 65 without EIC. Predictors of distant recurrence in multivariate analysis included final margin status ($p = .003$), pathologic node status ($p = .02$), and age less than 50 ($p = .04$). Patients with negative and close margins had a similar probability of distant metastasis.

Conclusion: Pathological margin status is the most important predictor of local recurrence following breast conservation with radiation. Patient age and EIC were significant predictors of residual disease at re-excision, and borderline significant predictors for local recurrence. Margin status was also a predictor of distant recurrence, with indeterminate or positive margins carrying a significantly increased risk.

450 Radiofrequency ablation of early-stage invasive breast tumors: results of a multi-center study.

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Purpose: The purpose of this study was to test the feasibility of using radiofrequency ablation (RFA) for the treatment of small (2.0 cm or less) invasive breast cancers.

Patients and Methods: Thirty three patients with early stage breast cancer were enrolled in the study at 3 treatment centers (M.D. Anderson Cancer Center, New York Weill Cornell Medical Center, John Wayne Cancer Institute). To be included in the study, patients had to meet the following criteria: (1) invasive breast cancer ≤ 2.0 cm; (2) tumor clearly identifiable by ultrasound; (3) no direct tumor involvement with overlying skin; (4) tumor not within 1 cm of skin or chest wall. Each patient was evaluated by ultrasound for determination of tumor size and tumor distance from the skin and the chest wall. Of the 33 enrolled patients, 30 received RFA of the primary tumor. During the procedure, tumor localization and assessment of the RFA process were performed with ultrasound. All patients subsequently underwent surgical excision of the ablation site. The specimens were examined histologically using H&E and NADH-diaphorase staining to assess the extent of coagulative necrosis.

Results: Complete ablation of the lesions visualized on ultrasound was achieved in 93% (28/30) of the cases. However, in two of these cases sonographically occult invasive carcinoma was found beyond the ablated lesion at pathologic examination. Two other tumors were not accurately localized under ultrasound resulting in incomplete ablation. One patient experienced a minor skin burn that was removed in the subsequent lumpectomy. No other minor or significant adverse events were reported.

Discussion: Provided that patients are carefully selected, RFA can successfully treat small invasive breast cancers, and may have the potential to replace surgical resection for the local treatment of these tumors.

451 Radiofrequency ablation of invasive breast cancer followed by delayed surgical excision.

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Background: Radiofrequency ablation (RFA) is gaining acceptance as a treatment modality for several tumor types, although its use in breast cancer remains investigational. This study was undertaken to determine the feasibility of treating small breast malignancies with RFA under local anesthesia and to evaluate the post-ablation MRI and histologic findings.

Methods: Patients with core needle-biopsy proven invasive carcinoma (less than 2 cm in greatest dimension) underwent an ultrasound-guided RFA procedure performed in an office setting. Surgical excision was undertaken 1-3 weeks later. All patients had a breast MRI performed before ablation and repeated within 24 hours prior to surgery.

Results: Ten (10) patients underwent the RFA procedure using a 2 cm array probe (Radiotherapeutics, Inc; Sunnyvale, CA). Mean tumor size was 1.2 cm (range 0.8-1.6 cm), measured by pre-ablation ultrasound. All patients completed the treatment with minimal or no discomfort. Time of RFA application ranged from 7 to 21 minutes (mean 13.8 min). There were no treatment-related complications other than minimal breast ecchymosis. Pre-RFA MRI showed enhancing tumors in 9/10 (90%) patients. Post-RFA MRI revealed no residual lesion enhancement in 8 of these 9 patients (89%), and the "zone" of ablation was demonstrated in all patients. One patient had enhancement at the anterior edge of the treated tissue consistent with residual tumor outside of the zone of ablation. Histologic evaluation of the ablated lesions removed at surgery revealed a spectrum of changes ranging from no residual tumor to coagulation necrosis with recognizable malignant cells. Immunostains for cytokeratin 8/18 were negative in these recognizable malignant cells, despite intense staining in the epithelial cells outside the treated area, suggesting non-viability. The patient with post-ablation MRI enhancement showed viable tumor cells outside of the zone of ablation, correlating with the MRI findings.

Conclusions: Radiofrequency ablation of small breast malignancies can be performed under local anesthesia in an office-based setting. Post-ablation MRI appears to predict histologic findings, although tumor viability needs to be assessed in a long-term study.

452 Targeted intraoperative radiotherapy (TARGIT) for breast cancer: an international trial.

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Breast conserving surgery followed by whole breast radiotherapy (including a tumour bed boost) is the current gold standard. However, symptomatic local recurrence occurs usually at the site of primary tumour, suggesting that it may be unnecessary to irradiate the whole breast in all patients. Also, up to 50% of local recurrence is attributable to a 'geographical miss'. We have pioneered* the use of targeted intra-operative radiotherapy (Targit) delivered to the tumour bed under direct vision in a standard operating room using a portable device (Intrabeam, Pec) that delivers soft x-rays (50Kv) from the surface of a spherical applicator, which is available in various sizes and is inserted into the tumour bed after wide local excision. The physical dose is 20Gy at the surface of the applicator and 5Gy at 1cm depth. The estimated biologically effective dose at the surface is 50-120Gy, with a high therapeutic ratio between tumour bed and skin, lung or heart dose; these can be further protected by thin tungsten impregnated sheets. The UK pilot study using this technique (n=25) as a boost was encouraging* with no recurrences and good cosmesis achieved at a median follow up of 34 months. The US (39) and the Australian (15) centres have treated 54 patients in local feasibility studies. We have begun a multicentre randomised trial to test this novel approach. The UK centre has randomised 29 patients(15 IORT,14 PostopRT). Totally, 94 patients has been treated with the novel technique. The longest follow up is 45 months (median=18m). Our 3rd patient had skin necrosis due to the applicator surface being brought too close to it. After a modification, we have had no further toxicity. In the randomised UK patients there was one local recurrence at 21 months. This was a protocol violation; she had involved margins but did not have re-excision. The trial is open for participation from interested centres. Targit has the potential a) to substitute the 6-wks of postoperative radiotherapy in low risk patients and b) given as an accurate and timely boost, to reduce local recurrence in high risk patients.

453 Paget's disease of the nipple: is breast conserving surgery an appropriate treatment?

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Background: The treatment of Paget's disease by mastectomy has recently been challenged in favour of breast conserving techniques. A large series of patients treated with mastectomy has been reviewed to assess the feasibility of less radical surgery.

Methods: 70 women with confirmed diagnosis of Paget's disease were reviewed. The type, grade, receptor and node status, mammography and pathological extent of the underlying breast malignancy were determined. The survival of patients with invasive disease was compared with matched controls without Paget's disease.

Results: The underlying malignancy was invasive in 58%. Despite the fact that only one third presented with a palpable mass, the malignancy was frequently extensive, being confined to the retro-areola region in only 25%. The true extent of the disease was underestimated by mammography in 55% of cases. 96.5% and 100% of DCIS and invasive disease respectively were of high cyto-nuclear grade. Over-expression of the c-erb-B2 receptors was detectable in 83% of invasive cancer and patients with Paget's disease had a significantly worse survival than matched controls. This difference appeared to be as a result of the c-erb B2 over-expression.

Conclusion: Paget's disease is often associated with extensive underlying malignancy, which is difficult to accurately assess either clinically or mammographically. As a consequence cone excision of the nipple-areola would have results in incomplete excision of cancer in 75% of cases. The underlying disease is of high grade and frequently over-express, c-erb B2 receptors with a resulting poor prognosis. Aggressive local and systemic treatment therefore would be merited.

454 Factors predictive of nipple involvement in breast cancer.

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Preservation of the nipple-areolar complex is one of the options frequently used in breast conservation surgery in patients with breast carcinoma. The challenge, however is to be certain that this complex does not harbor malignant cells, which could increase risk for recurrence. In this retrospective study, we analyze the parameters that play an important role in predicting nipple involvement. A total of 675 mastectomy cases performed in our institution for breast carcinoma were reviewed. The nipple-areolar complex had been sampled as per protocol to include the entire nipple, nipple base and two additional blocks of tissue beneath the nipple base. Cases were deemed to have nipple involvement if they showed invasive carcinoma (IC), in situ carcinoma (CIS), stromal or dermal lymphatic invasion in any of these sections. The parameters assessed for correlation included: age of patient, type of tumor, grade, size, presence of lymphatic/vascular invasion, dermal lymphatic invasion, lymph node status, extranodal invasion, presence and size of CIS, and immunohistochemical markers ER, PR, Her-2 and p53. Nipple-central duct involvement was seen in 22% (150/675) of mastectomies. In univariate analysis the following parameters had statistical significance: histologic type of IC (p=0.002); IC grade (p=0.0011); IC size (p=0.0218); lymphatic/ vascular invasion (LVI) (p=0.0001); dermal/lymphatic invasion (DLI) (p=0.0001); extranodal invasion (ENI) (p=0.0001); lymph node (LN) positivity (p=0.0001) and CERB 2 positivity (p=0.0086). The patients' age, presence or absence of CIS and size of CIS showed no significant association. In multivariate analysis of 675 cases, the following parameters retained significance: IC type (p=0.0001); IC grade (p=0.0112); IC size (p=0.0107) and DLI (p=0.0001). Her-2 expression (p=0.0019) and LN positivity (p=0.0004) were also found to be significant when the multivariate analysis was restricted to 561 cases, where all the data points were available. It is thus possible to predict the likelihood of nipple involvement based on the morphological features of the tumor and its immunoprofile.

455 African-American race does not impact local recurrence risk following breast conservation therapy in the community setting.

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Background: African-American race (AA) predicts adverse prognostic features in invasive breast cancer (IBC) and may be an independent risk factor for biologic aggressiveness. While previous studies note most factors influencing local recurrence (LR) following breast conservation therapy (BCT), lumpectomy and radiation, are independent of those predicting metastatic spread, few report impact of race on LR following BCT. We review BCT results for African-American Women (AAW) to determine if AAW have an increased risk of LR following BCT.

Materials and Methods: From 4/1/85- 4/1/01, 641 white women (WW) and 92 AAW, AJCC Stage I or II, underwent BCT for IBC. Median age was 60; 62 (26-92) for WW and 49 (24-83) for AAW ($p<0.05$). Because of the significant age difference, prognostic factors were stratified by age ≤35, 36-50, and >50. All received 45-54 Gy tangent breast irradiation with a 10-16 Gy boost. Median followup is 48 months (range 12-186). Family history, age, tumor size, adverse pathology, nodal status, receptor status, chemotherapy use and delay of radiotherapy >16 weeks were analyzed according to WW vs AAW overall and by age group.

Results: AAW were younger and presented with worse prognostic features overall ($p<0.05$), including larger tumor size, adverse pathology, positive nodes, and receptor negative tumors, but only adverse pathology remained significant after age stratification. For WW and AAW, overall ten-year freedom from LR (96% vs 95%), distant metastasis (DM) (94% vs 92%), and disease-specific survival (92% vs 98%) are not significantly different. Younger age and radiotherapy delay, but not race, were identified as independent risk factors for local recurrence on multivariate analysis; young age predicted DM. Breast recurrence did not predict DM.

Discussion: AAW with invasive breast cancer present at younger ages with more adverse pathology compared with WW, concordant with other reports. However, while AA race may independently predict more biologically aggressive tumors, race does not appear to impact risk of LR compared to WW. For properly selected women, BCT remains an appropriate choice for local control of IBC in AAW.

456 Cancer care providers appropriately offer breast conservation therapy (BCT): pilot data from the South Texas Veterans Health Care System (STVHCS).

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The specific aim of this analysis is to determine if BCT is appropriately offered to patients (pts) at the STVHCS. Inclusion criteria were patients with American Joint Commission on Cancer (AJCC) stage I/II and tumor size <5cm. Parameters assessed included surgical margins, adequacy of excision, # pts offered BCT, # pts receiving BCT, and #pts preferring mastectomy(M) to BCT. 53 cases were analyzed between 1990-2001 with the following AJCC stage classifications: 10 pts (19%) In-situ, 24 (45%) Stage I, 5 (9%) Stage II, 9 (15%) Stage III, 1 (2%) Stage IV, 5 (9%) Unknown Stage. 26 pts were excluded on stage and tumor size: 4 pt w/T>5cm, 11 with in-situ disease, 5 with >Stage II, 1 with no AJCC stage documentation, 5 with vague or unknown general documentation. Of the 29 with Stage I/II, 2 were further excluded due to tumor progression as a result of prolonged interval after diagnosis and a second because only an excisional biopsy was performed. Of the 27 eligible pts, none had diffuse micro-calcifications on mammogram, more than 1 lesion, chest wall or skin extension. All had negative surgical margins and adequate excision including axillary contents. BCT was offered to 23 (85%), was not or was poorly documented for 2 pts (7%) & not offered to 2 pts (7%) due to palpable lymph nodes in the axilla despite the fact that this finding is not considered a contraindication to BCT. 52% (12/23) of the cohort pts received BCT (1994=2, 1995=1,1999=1, 2000=5, 2001=3). 48% (11/23) opted for modified radical M over BCT. None of the 14 eligible pts lived more than 50 miles from a radiation therapy facility. The findings suggest STVHCS is appropriately offering BCT to eligible pts. Further, the data suggests that cancer care providers should be encouraged to document when BCT is offered, why BCT is not offered and the pt's ultimate treatment preference.

457 Long term radiation (RT)-induced cardiac perfusion defects following left sided tangential breast/chest wall irradiation.

Prosnitz R, Zhou S, Yu X, Hardenbergh P, Tisch A, Blazing M, Borges-Neto S, Wong T, Marks L. Duke University, Durham, NC

Background: We've previously demonstrated RT-induced perfusion defects 6-12 mos. after left-sided breast/chestwall RT(IJROBP 49:1023,01). We herein report on patients (pts) followed up to 24 mos. Methods: From '98-'01, 114 pts were enrolled on a prospective study to assess RT-induced changes in regional cardiac perfusion. Patients had pre-RT and serial post-RT evaluations of cardiac perfusion with single photon emission computed tomography perfusion scans (SPECT) 6, 12, 18, and 24 mos post-RT. Changes in SPECT scans were quantified in two ways: 1) A 12 segment summed rest myocardial perfusion score (SRS) grading the extent/severity of perfusion abnormalities. 2) Registering the serial SPECT scans with the 3D RT dose distribution to relate regional perfusion changes to regional RT dose. Results: The mean SRS pre-RT, 6, 12, 18 and 24 mos post-RT were 0, 1.7, 1.6, 1.9 and 2.1, respectively (based on 80, 47, 23, and 9 patients at each time point). The corresponding numbers for the pts with >5% of the LV within the RT field were 0, 4.7, 3.1, 3.3 and 4.1. Regional perfusion changes up to 24 mos post-RT were related to regional RT dose in 13 pts. Perfusion was reduced by 3-10% and 12-43% for regions of heart receiving ≈ <45Gy and >45Gy, respectively. For regions receiving <45Gy, perfusion reductions seen at 6-12 mos resolved by 18-24 mos. For regions >45Gy, however, the abnormalities identified at 6-12-mos post-RT improved but persisted at 18-24-mos. evaluations. Conclusion: RT-induced changes in cardiac perfusion are dose, volume and time dependent. Based on the SRS, RT induced perfusion changes appear to persist for at least 24 mos. Quantitative measures of cardiac perfusion suggest that reductions in cardiac perfusion may be reversible after low dose RT (<45Gy) but persist at higher doses. Longer follow-up and larger numbers of pts are needed to determine if these conclusions regarding changes in cardiac perfusion are valid and to see if they are associated with changes in global cardiac function and the development of clinically significant cardiac symptoms.

Acknowledgement: Funded in part by the grant DAMD 17-98-1-8071 and BC010663 awarded by the DOD.

458 Symptomatic "cardiac" events following radiation therapy (RT) for left-sided breast cancer: possible association with RT-induced changes in regional perfusion.

Yu XL, Prosnitz R, Zhou SM, Hardenbergh P, Tisch A, Blazing M, Borges-Neto S, Hollis D, Wong T, Marks L. Duke University Medical Center, Durham, NC

Background: Our group has previously demonstrated that tangential RT to the left breast/chest wall causes perfusion changes in the anterior myocardium(IJROBP 49:1023,01). We herein assess if RT-induced perfusion changes are associated with the development of symptoms consistent with cardiac dysfunction. Methods: Between '98-'01, 98 patients were enrolled into an IRB-approved prospective study and had pre and serial post-RT(6-24 mos) single photon emission computed tomography(SPECT) scans to assess changes in regional cardiac perfusion. Fifteen patients with abnormal pre-RT SPECT scans were excluded. The incidence of "cardiac" symptoms in patients with and without RT-induced perfusion defects were compared using a two tailed Fisher's Exact test. Results: With a median follow-up of 16 mos (range 6-24 mos), 10/83 evaluable patients had at least one episode of transient chest pain, occurring 0-14 mos(median 6.5 mos) post-RT. The rates of chest pain in the patients with and without a new perfusion defect were 9/31 and 1/52, respectively (p=0.001). A similar result was found when patients were segregated based on the use of chemotherapy. Cardiology evalution in these ten cases lead to a diagnosis of pericarditis in two, and the etiology of the pain remains unexplained in eight. None had myocardial infarction or congestive heart failure. Conclusion: "Cardiac" symptoms occur more frequently in patients with perfusion abnormalities by SPECT after RT than in patients with normal SPECT scans, suggesting that such perfusion defects may be clinically significant. However, other explanations remain possible. Women who know that they have RT-induced perfusion defects may be more likely to seek medical attention for episodes of chest pain. Alternatively, perfusion defects may simply correlate with an increased rate of pericarditis in patients with larger irradiated heart volumes. Since the number of observed episodes of symptoms remains small, additional follow up with larger numbers of patients will be necessary to better assess the clinical significance of RT-induced perfusion defects.

Acknowledgement: Funded in part by grant DAMD17-98-1-8071 and BC010663 awarded by the DOD, PLUNC planning software supported by UNC.

459 Myocardial perfusion changes in patients irradiated for left-sided breast cancer and correlation with coronary artery distribution.

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Purpose: To evaluate post-irradiatory regional heart perfusion changes with single photon emission tomography myocardial perfusion imaging (SPECT) in 69 patients treated with tangential photon beams radiation therapy (RT) for left-sided breast cancer. To correlate SPECT changes with percent irradiated left ventricle (LV) volume and risk factors for coronary heart disease (CHD).

Methods and Materials: Rest SPECT of the LV was acquired pre-RT and at 6 mos. intervals post-RT. The extent of defects (%) with a severity greater than 1.5 SD below the mean was quantitatively analyzed for the distributions of the left descending (LAD), left circumflex (LCX), and right (RCA) coronary arteries based on computer assisted polar map reconstruction, i.e bull's-eye-view. Changes in perfusion were correlated with percent irradiated LV receiving more than 25 Gy (range: 0-32%). Data on patient- and treatment-related factors were collected prospectively, e.g. cardiac pre-morbidity, risk factors for CHD, chemotherapy-/hormonal treatment.

Results: In the LAD distribution, there were increased perfusion defects at 6 mos. (median: 11%; IQR:2-23) compared to base-line (median: 5%; IQR: 1-14) (P<0.001). There were no increases in perfusion defects in the LCX- or RCA-distributions. In multivariate analysis, the SPECT perfusion changes in the LAD-distribution at 6 mos. were independently associated with percent irradiated LV (P<0.001), hormonal therapy (P=0.005), and pre-RT hypercholesterolemia (P=0.006). The SPECT defects in the LAD distribution at 12 and 18 mos. were not statistically different from those at 6 mos. The perfusion defects in the LAD distribution were limited essentially to the regions of irradiated myocardium.

Conclusion: Tangential photon beam RT in patients with left-sided breast cancer was associated with short-term SPECT defects in the vascular distribution corresponding to the radiation portals. Factors related to the extent of perfusion defects included the percent irradiated LV, hormonal treatment, and pre-RT hypercholesterolemia.

460 Customized blocking of tangential radiation (RT) breast fields: excluding the heart vs. covering the breast. Striking a balance.

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Purpose Standard left-sided RT tangential breast fields often include a portion of the heart that may be clinically significant. Our practice thus is to often use a heart block, designed using CT-based 3D imaging. This technique often leaves a small portion of breast tissue untreated. We herein quantify the percentage of breast untreated with this technique.

Methods: 23 patients with left-sided breast cancer treated with lumpectomy and tangential RT, were studied using the PLUNC 3D planning system. Glandular breast and heart tissue was identified on serial axial CT images. Three competing plans were generated for each case: midline tangents with no heart block; the same tangents with a complete heart block defined using beams-eye view-tools; and an intermediate compromise between these two. For each of the plans the % of heart receiving >=20% and >=50% and the % of breast receiving >=75% and >=90% of the prescription isodose was calculated and compared using a 2-tailed t-test.

Results:

Table 1. Dosimetric Comparisons of Blocking Strategies

Parameter	Midline Tangents	Compromise Block	Full Heart Block	P value, midline vs. compromise block	P value, midline vs full heart block
% Breast getting at least 75% of dose	93.6	92.9	91.9	p=0.008	p<0.001
% Breast getting at least 90% of dose	84.1	83.4	81.4	p=0.008	p<0.001
% Heart getting at least 20% of dose	5.8	2.0	0.13	p<0.001	p<0.001
% Heart getting at least 50% of dose	3.9	0.85	0.04	p<0.001	p<0.001

Midline tangents blocked on average 3.9% more of the heart from getting >=50% of the dose (range 0-9%). A full heart block typically omitted on average 2.7% more breast tissue from getting >=90% of the dose (range 0-12%).

Conclusion: A heart block in a left-sided tangent fields effectively eliminates incidental cardiac irradiation, but does reduce dose to a portion of the breast. However, since the % of untreated breast is small, and typically located in the far medial/inferior breast, far from most tumor beds, this is unlikely to compromise cancer control.

The authors thank the University of North Carolina at Chapel Hill for use of the PLUNC (Plan-UNC) system.

Research supported in part by a grants from the Armstrong Family Foundation and DAMD# 17-98-1-8071.

461 Influence of radiotherapy delivery and adjuvant treatment on local relapse in early breast cancer patients: 7-year follow-up results of French Adjuvant Study Group (FASG).

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Purpose: To determine if the moment of radiotherapy (RT) delivery modified the incidence and outcome of local relapse (LR) after lumpectomy, and to identify prognostic factors of LR in early, node-positive (N+) breast cancer (BC) patients (pts).

Patients and methods: Among the 8 FASG trials, 1800 N+ pts received RT after lumpectomy: 474 pts had RT after surgery, followed by hormonotherapy (HT) in 379, or no systemic treatment in 95; 567 had RT after the 3rd chemotherapy (CT) cycle (cy) in pts receiving either 1-3 CT cy (250) or 4-6 CT cy (317); 759 pts had RT after the 6th CT cy. For the 1326 pts receiving chemotherapy (CT), it was FEC regimens (epirubicin 50,75, or 100 mg/m^2) in 84% of pts, and other epirubicin (EPI)-based CT in others. The median follow-up was 7 years. Multivariate analysis was performed in the whole population and in subgroups of pts receiving either CT alone, either CT and HT, or HT vs not.

Results: LR occurred in 29 pts (6.1%) if RT delivered after surgery, 98 (17.3%) after 3rd cy, and 71 (9.4%) after 6th cy. In the whole population, moment of RT was not a prognostic factor of LR, and the main prognostic factors were age < 40 yrs (relative risk [RR] = 1.54), SBR grade 2-3 (RR = 1.96), no HT (RR = 2.70), and 1-3 CT cy (RR = 1.59). In pts receiving CT alone, prognostic factors were age (RR = 1.54), number of CT cy (RR = 1.53). In pts receiving CT and HT, the only prognostic factor was hormone receptors (HR) status (RR = 4.10 for HR- pts). Finally, HT improves significantly LR for HR+ pts, with or without CT combined (RR = 0.39 and 0.58, respectively).

Conclusion: In BC pts treated by primary conservative surgery, a delay in RT delivery after the end of CT does not significantly increase the incidence of local relapse. These results are consistent whatever the groups of pts studied. The main prognostic factors seem to be young age, poor histoprognostic grade, and the adjuvant systemic treatment.

462 Comparison of axillary failure rates following sentinel lymph node dissection (SND) alone versus SND followed by axillary dissection (SNAD) in patients with early stage breast cancer treated with tangential breast irradiation without an axillary field.

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Background: The axillary failure rate in breast cancer patients (BCP) treated with lumpectomy, axillary lymph node dissection, and radiation therapy (RT) to breast tangents alone is less than 2%. This resulted in the elimination of axillary RT in BCP with negative lymph nodes, minimizing patient morbidity. There are no studies documenting the rates and patterns of axillary failures in BCP treated with SND only followed by breast tangent RT while excluding the axillary field.

Materials & Methods: A retrospective analysis of 101 BCP treated with either SND or SNAD followed by RT at Thomas Jefferson Univ. from 1997 to 2001. There were 48/101 of patients treated with SND only, and 53/101 treated with SNAD. The mean follow-up of the BCP was 33 months (range 6-45). The two BCP cohorts were matched for age, stage, technique of sentinel lymph node examination, follow-up, grade, margins and menopausal status. Patients were treated with RT to breast tangents only to a median dose of 46 Gy followed by a 16-20 Gy electron boost. All patients were planned using CT simulation which was designed to exclude coverage of the level 1 axillary lymph nodes.

Results: There were 0/48 of patients treated with SND followed breast tangent RT only that experienced a local axillary failure. Similarly, we found 0/53 SNAD patients developed metastatic axillary disease following completion of breast conserving therapy. The two BCP cohorts experienced similar low rates of axillary failures (p=NS). To date, the rates of lymphedema in both cohorts are similar and longer follow-up may be required to establish the long-tern advantage of SND. As of 6/02, no patients treated with SND followed by breast tangent RT developed metastatic disease and all patients are alive and NED.

Discussion: This study provides early evidence that BCP treated with SND followed by RT to breast tissue while omitting the axillary field have low rates of axillary failures. These findings will reassure physicians that eliminating the treatment of the axillary field in patients with negative sentinel lymph nodes without a complete axillary dissection will provide excellent long-term cure rates.

463 Complication rates following breast reconstruction with and without radiotherapy.

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Background: Existing data on the effect of radiotherapy (RT) on reconstruction (recon) outcome varies. Therefore, we reviewed outcome after recon and RT vs. recon without RT to attempt to identify features with most favorable complication profile.

Methods: Records of patients that had received recon with or without RTwere retrospectively analyzed for recon type (primarily expander/implant (E/I) or TRAM), sequencing of RT and recon, complications, and cosmesis. Categorical associations were examined by Fishers exact test. Logistic regression was used to examine the predictive power of covariates on complications, wound healing and poor cosmesis.

Results: Of 152 patients (23% non-white), 48 received RT, 104 did not. Consistent with previous reports of high complication rates with E/I and RT, patients who received RT were more likely to have TRAM (N=29) as opposed to E/I (N=19), while patients treated without RT mere more likely to have E/I (59/104) (p=0.056). Sequencing also differed; patients without RT were more likely to have immediate recon, whereas patients with RT delayed (p=0.034). Patients treated with delayed recon, were more likely to receive a TRAM if they were irradiated, and E/I if not (p= 0.036). The overall complication rate was higher in patients who received RT (19/48, 40%) as oppose to those who did not (25/104, 24%) (p=0.05). However, the difference was entirely attributable to an increase in minor wound healing complications (e.g. seroma) (p=0.02). In univariable and multivariable logistic regression modeling, we failed to show predictive ability for recon type or sequence on complications for the entire group or the subset who received RT. The same was true for other covariates and the outcomes of poor cosmesis and major and minor wound healing, with the exception of an increase in complications in minor wound healing of those who received RT (p=0.02). This result however, was driven by a total of 8 patients.

Conclusions: In our series, major complications were not significantly increased in patients treated with RT. Recon type or sequencing did not correlate with complications, except for possible slight decrease in minor complications in patients treated with immediate TRAM recon.

464 Definition of post-lumpectomy (L) tumor bed for radiotherapy (RT) boost field planning: computed tomography (CT) versus surgical clips.

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Background: Following L plus breast RT, a tumor bed boost improves local control. Locating the tumor bed is crucial for accurate boost planning. We herein compare the location and extent of the tumor bed as defined by surgical clips and CT scans. **Methods:** Charts and planning CT images of 31 operated breasts in 30 patients (pts) with invasive (N=25) or DCIS (N=6), stage T1-2 N0-1, who underwent L between 2000-02 were reviewed. Pts had one (N=15) or multiple clips (N=16) placed in the L cavity. For each pt, serial CT images (5 mm spacing) were used to measure the depth, the transverse (medial-lateral) and the longitudinal (superior-inferior) dimensions (multi-clip), and geometric center of the tumor bed defined independently both by the clips (clip-bed) and by the CT (CT-bed; based on tissue density). For each patient, the clip- and CT-based measurements were compared. The ability to readily identify the tumor bed on CT was correlated with various factors, such as age, total breast tissue excised, and the time interval between L and RT. **Results:** 1. Maximal tumor depth (all pts): CT and clip measurements were typically identical; except in 3 pts (10%) where the CT-bed was 45, 12, and 5 mm deeper than the deepest clip. 2. Transverse/Longitudinal extent (multi-clip pts): The CT-bed extended beyond the clips by: -6 to 27mm (mean 7) medially, -10 to 37mm (mean 6) laterally, -15 to 25mm (mean 0) superiorly and 0-20mm (mean 4) inferiorly (minus sign indicates extension of clip beyond CT-bed). 3. Tumor-bed center (all pts): CT- and clip-based measurements differed by 2-37 mm (mean 6) and 1.5-25mm (mean 6) in the transverse and longitudinal directions, respectively. The CT-bed was more readily visible in patients with a shorter time interval between surgery and RT. **Conclusions:** The maximal depth of the tumor bed was similar with the two methods. The extent of the tumor bed however, and the centers of the clip- and CT-beds can differ significantly. This may indicate an underestimation of the tumor bed as defined by the clip(s) only, and justifies integration of CT information in boost field planning, especially when only a single clip is inserted. The utility of CT in this regard appears to diminish with time following surgery.

465 Improving dose homogeneity with intensity modulated tissue compensation in breast irradiation.

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Purpose: There is developing clinical interest in using IMRT technology to improve dose homogeneity in treatment of the intact breast. In this report we compare the dosimetry of standard wedged tangents and combined wedged/ IMRT tangents, based on twenty patients treated at our institution.

Materials and Methods: Twenty consecutive patients treated post-lumpectomy with adjuvant radiation therapy were selected for the study. All patients were treated with 60 Gy in 30 fx, with IMRT tangents followed by an electron boost, on a Varian 2300cd or 600cd with dynamic MLC. A GE Lightspeed CT scanner was used for simulation, and beam geometry was done with GE AdvantageSim. The plan was imported into CadPlan with Helios IMRT. A standard wedged tangent plan and a combined plan with the electronic tissue compensator added were created. We defined two points to quantify off-axis doses in the deep breast, superiorly and inferiorly, as 3 cm from the top and bottom of the tangent fields, at midplane, 1 cm anterior of the lung/tissue interface. The plans were analyzed for the percent of prescribed dose delivered at these points in the superior and inferior breast.

Results: Mixing tissue compensated beams with traditional wedged tangents improves coverage of the deep superior breast relative to standard treatments. The mean point dose was higher, closer to prescription dose, in the superior breast with the combined plans (98.3%, std 0.9) relative to the traditional wedged plans (95.1%, std 2.8), for 17 of the 20 patients (mean difference 3.2%, std 2.4, $p<0.001$, paired t-test). In addition, the combined plans reduce overdosing in the inferior breast. The mean point dose was lower, closer to prescription dose, in the inferior breast with the combined plans (100.2%, std 1.2) relative to the traditional wedged plans (102.9%, std 3.0) for 18 of the 20 patients (mean difference 2.7%, std 2.8, $p<0.001$).

Conclusion: Adding intensity modulated tissue compensation in breast irradiation can improve dose homogeneity, particularly off-axis in the superior and inferior breast, where traditional wedged tangents show variation in dose. We have treated over eighty patients using this technique with good cosmetic results.

466 IMRT removes the dose hot spots induced by conventional wedged irradiation technique for adjuvant breast irradiation: potential impact on acute skin toxicity.

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Background: The conventional wedged breast irradiation (WT) is associated with a significant rate of acute skin toxicity (AST) ranging between 36 to 50%. It has been demonstrated that important predictor of AST are the presence of dose variation in excess of 10% within the breast (RR=9.7) and the breast size (RR=5.7). The skin toxicity occurs most frequently in the inframammary fold and in the axilla, where conventional WT produces hot spots. Intensity Modulated Radiation Therapy (IMRT) is a technique that could compensate for tissue missing by superimposing a large quantity of small "segmented" fields. Methods: A dosimetric study was performed on a cohort of 25 consecutive breast cancer patients referred at TSRCC for adjuvant breast irradiation. WT and IMRT plans were generated for each patient following CT simulation. Dose distribution homogeneity was evaluated on Differential Dose Volume Histograms (DDVH) and on a Sagittal Dose Gradients (SDG).

Results: 1/- All patients planned with the conventional WT demonstrated significant hot spots in the axillary tail and in the inframammary fold of the breast. In the inframammary fold the average SDG was 10% (range: 2% to 20%), while 60% of the patients had hot spots in excess of 10%. The SDG was found more important for large breast sizes ($p<0.02$). 2/- IMRT improved dose homogeneity compared to WT as measured by an average increase in the DDVH peak height by 70%. IMRT consistently reduced the hot spot situated in inframammary fold from mean values of 10% to 0.4% ($p<4.10\text{-}9$). This reduction is more important for large breast patient.

Conclusions: The standard WT produces large dose variation within the breast, and this effect is more pronounced for patient with larger breast sizes. The ability of breast IMRT to improve dose distribution homogeneity suggests that AST may be significantly reduced.

467 Breast radiation in the prone position for large pendulous breasts after breast conserving surgery: analysis of radiation dose distribution.

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Background: Large pendulous breasts have been considered a relative contraindication to breast conserving therapy (BCT) in early stage breast cancer patients due to potential difficulties delivering breast radiation (BR). Significant radiation dose heterogeneity can occur in large breasts causing more late toxicity and poorer cosmetic outcomes. We have begun BR in the prone position (PP) using 3-dimensional treatment planning techniques in an attempt to improve dose homogeneity and minimize poorer cosmetic outcomes.

Material and Methods: For 15 of the initial patients, treatment planning records were retrieved and reloaded in the CMS FOCUS® software. The following parameters were evaluated for each plan: dose volume histogram (DVH) of the target breast volume (TBV) at 100%, 110% and 125% of the prescription dose (RxD), DVHs of the ipsilateral lung receiving ≥ 20 Gy, and heart at 50% prescription dose. Four indices were piloted to evaluate dose homogeneity across the TBV and 4 smaller reference volumes at -0.5 cm, -1.0 cm, -2 cm, and -4 cm: DVH RxD/ DVH 95% RxD, DVH RxD/ DVH 105% RxD, DVH RxD/DVH minimum dose to 99%of volume, and DVH RxD/ DVH maximum dose to 1% of volume.

Results: The RxD was delivered to a mean of 68% (41-91%) of the TBV. The DVH 110% RxD was 0 for 6, and the other 9 had a mean DVH of 10% (1-32%). The DVH 125% was 0 in all cases. The DVH lung ≥ 20 Gy was 0 for 10 (67%) and a mean of 6% (1-14%) for 5. For the 10 left-sided cases, the DVH heart 0.5RxD was 0 for 7. The first 3 indices demonstrated relative homogeneity across the different reference volumes of the breast. For the fourth, the relative %breast volume receiving the maximum dose increased for the smallest reference volume.

Conclusions: BR in the PP for BCT in early stage breast cancer delivered the RxD + 5% homogeneously with exclusion of the lung and heart (left-sided cases) for the majority of patients. The newly defined breast homogeneity indices for radiation dose provide a benchmark for comparison to cases treated supine. Future studies will correlate degree of dose homogeneity with toxicity and cosmetic outcomes from BR in the PP.

468 Taxane chemotherapy in sequence with breast radiotherapy results in a low risk of pneumonitis.

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Purpose: The use of Taxol chemotherapy in sequence with either breast radiotherapy has been reported to have an increased incidence of pneumonitis.1 In our clinical practice we have not experienced an increased incidence of pneumonitis in patients who have been treated with taxane chemotherapy (Taxol and Taxotere) and breast radiotherapy.

Methods and Materials: The authors retrospectively reviewed charts of patients who were treated with taxane based chemotherapy regimen followed by radiotherapy to the breast.

Results: 47 patients were treated from 8/95 to 8/01. Patients had a median of 4 cycles (range 1-15). 1 patient received 15 weekly cycles, 44 patients 4 cycles, 1 patient 3 cycles and 1 patient 1 cycle of chemotherapy. 9 patients received Taxotere chemotherapy and 38 Taxol chemotherapy. All patients also had Adriamycin and cytoxan chemotherapy. 15 patients were treated in a neoadjuvant setting. Their median interval from the end of chemotherapy to radiotherapy was 49 days (range 13-128). For patients treated adjuvantly the median interval was 74 days (range 21-146). Median dose of radiotherapy to the breast was 50 Gy (range 45-54). 21 patients received a boost with median dose of 12.5 Gy (range 10 - 16). 3 patients were treated with supraclavicular fossa radiotherapy to 45 Gy. No patient received internal mammary radiation. The median age was 49 (range 29-70). Median follow-up was 32.5 months (range 3-77). Median tumor size was 14 mm (range 1-30). 36 patients were T1, 10 T2 and 1 not recorded. 12 patients were N0, 34 N1 and 1 not recorded. TNM stages were: I (12), IIA (27), IIB (7) and 1 not entered. 40 patients were ER positive, 6 negative and 1 was not recorded. In patients with positive lymph nodes the median number of positive lymph nodes was 2 (range 1-6). The median number of dissected lymph nodes was 10 (range 0 - 24). Histology was as follows: IDCA (43), ILCA (3) and MUC (1). There were no clinical incidents of pneumonitis in our patient group.

Conclusion: In our clinical practice we have not found an increased incidence of clinical pneumonitis in patients treated with breast radiotherapy despite the use of a Taxane based chemotherapy with as many as 4 cycles even when combined with adriamycin chemotherapy.

1Taghian AG et al. J Natl Cancer Inst 93:1806-1811.

469 COX-2 and HER-2 expression are related in ductal carcinoma in situ (DCIS).

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Background: Cyclooxygenase type-2 (COX-2) is over-expressed in malignant tumours including breast cancers, but the mechanism and stage of COX-2 up-regulation in carcinogenesis is unclear. Studies have linked over-expression of the oncogene HER-2 (*cerbB-2*) with induction of COX-2 in breast cancer cell lines. This study aimed to determine and compare COX-2 expression in normal breast, pre-invasive (DCIS) and invasive breast cancer (IBC) and in DCIS, to investigate the relationship of COX-2 expression to HER-2 over-expression, oestrogen receptor (ER) expression, tumour grade and cell proliferation.

Methods: COX-2 (scored 0 to 4, with ≥2= positive), HER-2 (scored 0 to 3, with ≥2= positive), ER and Ki67 (a marker of cell proliferation) expression were determined by immunohistochemistry on paraffin tissue sections. Antigen expression was assessed by light microscopy and a minimum of 1000 cells counted on each section.

Results: The level of COX-2 expression in DCIS ($p<0.001$*) and IBC ($p<0.001$*) was significantly greater than in normal breast. There was no difference in the level of COX-2 expression between DCIS and IBC ($p=0.87$*). In DCIS (n=102), 69% of tumours were HER-2 positive and 75% COX-2 positive. COX-2 expression was significantly higher in HER-2 positive DCIS ($p=0.05$*), but showed no association with ER status, cell proliferation or tumour grade.

COX-2 expression scored 0 (absent) to 4 (maximum) for each tissue

Tissue (number)	0	1	2	3	4	COX-2 +ve (≥2)	*p value
Normal breast (36)	21	12	3	0	0	9%	Reference
Normal around DCIS (46)	33	5	7	1	0	17%	0.44
Invasive cancer (54)	6	12	12	21	3	63%	<0.001
DCIS (102)	11	14	37	31	9	75%	<0.001

*Chi square test

Discussion: COX-2 is up-regulated in in-situ breast cancer. Signalling through the over-expressed HER-2 receptor may modulate COX-2 induction in DCIS.

470 Comedo ductal carcinoma in situ (DCIS) may be associated with worse outcome compared to other DCIS: Implications for sentinel node biopsy.

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Background: Current consensus is that all histologic types of DCIS should be managed conservatively because of excellent survival; axillary lymph node sampling is considered superfluous. However, "comedo" DCIS may have a worse outcome due to undetected microscopic invasive cancer that already has mutations that enable rapid proliferation and metastases. **Materials and Methods:** We conducted a retrospective study of survival for subsets of in situ breast cancer diagnosed during the years 1983-1998 using the tumor registry of Hoag Hospital. Comedo DCIS was designated in pathology reports by staff pathologists based on the presence of high-grade cellular features, the necrosis that characterizes this histologic subtype, and the absence of invasive cancer. Actuarial survival curves were calculated by Cancer Data Services using Electronic Registry Systems, Inc. software program. **Results:** There were 572 cases of in situ cancer identified: 70 lobular carcinoma in situ (LCIS), 103 comedo DCIS, and 469 other DCIS. Exclusion of 9 dual entries (a second diagnosis of in situ breast cancer) and 46 patients with a prior diagnosis of invasive cancer left for analysis: 55 LCIS (median age 51.0 years), 100 comedo DCIS (median age 56.5 years), and 417 other DCIS (median age 54.0 years). The first 5 years after diagnosis, there was one death in the LCIS group (1.4%), 8 in the comedo group (8.0%) and 15 in the remaining DCIS group (3.6%) [p=.041, Fisher's exact test for comedo vs other DCIS]. The observed 5-year survival rates were 98% for LCIS, 96% for non-comedo DCIS, 91% for comedo DCIS, and 87% for patients with localized invasive breast cancer [p=.057, log rank test for comedo vs other DCIS]. This difference was seen in spite of constraints of the state reporting system. The DCIS data set failed to capture patients who had DCIS but underwent an axillery node sampling procedure that disclosed lymph node metastases. **Discussion:** Two recent studies reported 12-13% frequency of sentinel node involvement in patients with high risk DCIS (Lauber-Demore, Am Surg Oncol 7:631-633, 2000. Cox, Am J Surg 67:513-519, 2001). Patients with comedo DCIS should undergo sentinel node biopsy to identify a subset with invasive cancer that may benefit from adjuvant therapy.

471 Male breast carcinoma: outcomes and predictors of local-regional failure in patients treated without radiation therapy.

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Background: Male breast cancer accounts for less than 1% of all breast cancers and less than 0.1% of all male cancer deaths. Approximately 1,200 new cases are diagnosed annually. There are however few data that provide insight into patients who might benefit from irradiation. This study is undertaken to ascertain the outcomes of male breast carcinoma patients who received treatment at The University of Texas M. D. Anderson Cancer Center (UTMDACC) without irradiation and to identify predictors of local-regional recurrence.

Patients and Methods: A total of 290 male patients registered at UTMDACC with a diagnosis of breast cancer between 1944 and 2000. Of those, 142 received treatment at our institution; 77 patients were not irradiated and were retrospectively reviewed. Available breast imaging was reviewed, as were pathology slides. Kaplan -Meier statistics were used for outcomes.

Results: The median follow-up time was 6.1 years (range 0.5 - 31.1), while the median patient age was 61 years (range 33 - 82). Mastectomy was performed in 59 patients (77%). Chemotherapy and/or hormonal therapy were administered to 53% of the patients. Of the patients receiving chemotherapy, 57% received adriamycin. Overall survival at 5 and 10 years respectively was 62% and 49%. Clinical T stage presentation was 3% Tis, 52% T1 or T2, and 27% T3-4. Almost half of all patients were clinical N0 (47%); 30% were clinical N1. Margins were positive, close, or unknown in 49%. The overall rate of local regional failure was 18.2% with the chest wall (87%) and supraclavicular area (33%) being most frequent. Margin status, tumor size, and number of positive axillary nodes predicted for LRF ($p \leq .05$); focal skin involvement did not.

Conclusion: Breast cancer in males is infrequent. Mastectomy is the current primary management of local disease. Margin status, tumor size, and number of positive nodes are predictive of patients who may benefit from irradiation.

472 Steroid hormone receptor expression in male breast cancer.

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About 1% of breast cancers occur in males, accounting for about 0.5% of all cancers in men (Jaiyesimi, 1992). Nearly all varieties of in situ and infiltrating female breast carcinoma also occur in males and generally with the same frequency. Estrogen receptor α (ERα), progesterone receptor (PR) and androgen receptor (AR) positivity is more frequent in breast carcinomas of the male, occurring in over 80% of tumours compared to 60% in females (Shoker, 1999. Rayson, 1998). As in the female there is a strong association between ERα positivity and response to endocrine therapy.

The aim of this study is to assess the expression of the steroid hormone receptors ERα, PR and AR and the less well characterised ERβ in male breast cancer. Immunohistochemical studies of ERβ expression in female breast cancer have found between 30 & 75% positivity using a variety of antibodies. In our hands 74% of female breast cancers are ERβ positive (Skliris, 2001). Immunohistochemical analysis of sections from formalin fixed paraffin embedded breast cancers was carried out. The antibodies ERα 1D5, PR PgR636 and AR 441 were employed in addition to, for ERβ, the monoclonal antibody 14C8 (Genetex, TX). This antibody has been validated by our group (Skliris, 2002). Eleven male breast cancers were stained. The tumours were mostly ductal, with two showing mixed ductal and lobular features, one lobular and one papillary tumour. Three tumours were grade 1, seven grade 2 and one grade 3. The Allred scoring system, was used to score all staining. Scores of 3 or above were positive.

73% of the cancers were ERα positive, 73% PR positive, 82% AR positive and 91% ERβ positive. ERα, PR and AR was restricted to epithelial cell nuclei. In contrast positive ERβ staining was observed not only in epithelial cell nuclei but also in occasional stromal and endothelial cells.

Our results suggest that, in addition to higher ERα, PR and AR expression, male breast cancers are also more likely to express ERβ than their female counterparts. The very high level of expression of steroid hormone receptors in male breast cancer suggests an essential role for hormones in this disease.

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473 In vivo imaging of angiogenesis in breast cancer xenografts during therapy.

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Introductions. The purpose of the present study was to develop a sensitive and accurate non-invasive in vivo imaging method for the early detection and monitoring of angiogenesis of breast tumors in mice.

Methods. Human breast tumor cells (MCF-7, T47D) were transfected separately with a replication deficient adenoviral (Ad) vector encoding GFP (Ad-GFP). At first a different number of GFP-positive tumor cells (5 - half a million cells) were implanted in nude mice to assess the sensitivity of light-based imaging. In nextl experiments, the GFP+ tumor cells were implanted in two groups (6/group) of nu/nu nude mice sc. The mice were imaged with a fluorescence stereomicroscope in 1 hour after cell implantation. In vivo images were collected in all live mice to detect GFP+ tumor cells and the development of angiogenesis. From fourth day, in one group of mice, anti-angiogenesis antibody DC101 was ip injected on every third day for 12 days. Images were collected on every third day on all mice. On 13th d, the tumors were removed aseptically and collected in sterile tissue culture media for further investigation.

Results. From the initial sensitivity test, GFP-positive tumor cells as small as 5 in number were visualized in live anesthetized mice by a light-based stereomicroscopy. In the angiogenesis studies, at 1 h, in all mice, sc tumors with highly brilliant green fluorescence were detected by optical imaging. However, the angiogenesis around the tumor regions was not detected until 2nd day for MCF-7 and third day for T47D bearing mice in vivo. SC images showed angiogenesis around the tumors in all control mice after third day for MCF7 and fourth day for T47D. However, in DC101-treated mice, angiogenesis decreased with time and dosage. Effects of therapeutic intervention by DC101 were visualized in live animals. Gradual decrease of angiogenesis, reduction of tumor size, and considerable decrease of GFP were captured in images over time. More importantly, the whole process did not require any surgical procedure or sacrificing animal . Conclusions. The present study provides the evidence for the application of fluorescent stereomicroscopy as an in vivo tool for the early detection and monitoring of angiogenesis of human breast tumors in mice models without surgery, with potential for investigating therapeutic interventions in humans.

474 Inflammatory breast cancer shows intense ongoing angiogenesis and strong E-cadherin expression.

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Introduction: Inflammatory breast cancer (IBC) is the most aggressive form of locally advanced breast cancer. Improved understanding of the mechanisms reponsible for the differences between IBC and non-IBC might provide novel therapeutic targets.

Patients and methods: We have studied 35 consecutive patients with clinically suspected and pathologically confirmed IBC, biopsied prior to the initiation of chemotherapy. All tumours were poorly differentiated carcinomas showing lymphovascular permeation. Angiogenesis was evaluated by Chalkley counting on CD34 immunostained slides, assessment of endothelial cell proliferation (ECP) on CD34-PCNA (proliferating cell nuclear antigen) double immunostained slides and of vessel maturity on CD34-a-smooth muscle actin double immunostained slides. The presence of fibrin, expression of the endogenous hypoxia marker carbonic anhydrase IX (CA IX) and E-cadherin expression were detected with the NYB.T2G1, M75, and HECD-1 monoclonal antibodies respectively. For comparison, the same parameters were obtained in a group of 104 non-IBC patients.

Results: Vascular density assessed by Chalkley counting was significantly higher in IBC than in non-IBC ($p<0.0001$), as was ECP ($p=0.01$). Although the mean % of immature vessels in IBC was 87.3 %, this was not significantly different from non-IBC. Abundant stromal fibrin deposition was observed in 29 % of IBC and in only 8 % of non-IBC ($p=0.02$). The expression of CA IX was significantly less frequent in IBC than in non-IBC ($p=0.046$). There was a significant positive correlation between the expression of CA IX and ECP ($p=0.04$), implying that the angiogenesis is partly hypoxia driven. However, the higher ECP in IBC and the less frequent presence of CA IX in IBC versus non-IBC, points at a role for other factors than hypoxia in stimulating angiogenesis. E-cadherin expression was found at cell-cell contacts in 100 % of the tumor cells in all but two IBC, one of the latter showing a lobular morphology. Strong expression of this cell adhesion molecule, known to be down-regulated in poorly differentiated non-IBC, was present in both lymphovascular tumoremboli and infiltrating tumor cells.

Conclusion: Both the intense ongoing angiogenesis and the strong E-cadherin expression could contribute to the highly metastatic phenotype of IBC.

475 Vascular endothelial growth factor (VEGF) fails to predict response to neoadjuvant chemotherapy for primary breast cancer.

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Background: Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis. Elevated tumour VEGF is a poor prognostic factor in breast cancer and correlates with increased tumour grade and size, a greater risk of relapse and worse survival.

Materials and methods: This study measured plasma VEGF before and during neoadjuvant anthracycline-based chemotherapy for primary operable breast cancer. Serial measurements of plasma VEGF were taken in 22 patients (median age 47, range 34-67) undergoing FEC chemotherapy for T2-4 N0 or N1 biopsy-proven breast carcinoma. Plasma VEGF was measured before the first, second, fourth and sixth cycles of treatment using a radiolabelled ELISA immunoassay. Baseline VEGF and changes in VEGF expression were correlated with clinical, radiological and pathological response to treatment.

Results: The median (range) plasma VEGF levels (pg/ml) before cycles 1, 2, 4 and 6 were similar: 39.0 (30.1 - 182.9), 39.1 (33.1 - 127.7), 44.2 (33.5 - 78.5) and 42.0 (29.8 - 73.0) respectively. There was little inter- or intra-patient variability (plasma VEGF measured between 30-50 pg/ml in 75% of cases). No consistent variation of VEGF with time could be demonstrated. There was no significant difference in baseline VEGF, or change in VEGF after one cycle of treatment, between responders and non-responders classified by clinical or radiological criteria. Pathological responders were more likely to have increased VEGF levels after one cycle of chemotherapy compared with non-responders ($p=0.048$).

476 Angiogenesis in a tumor tissue array system in breast cancer. Immunohistochemical analyses of VEGF, KDR and TSP-1 in relation to immunoassay evaluation of VEGF.

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Background: Angiogenesis is a prognostic indicator in breast cancer related by specific angiogenic factors and their receptors and inhibitors. Vascular endothelial growth factor (VEGF) is the most important angiogenic factor and it's receptor KDR is thought to be upregulated by VEGF. Recent data indicate that thrombospondin, TSP-1, considered an inhibitor of angiogenesis, may have proangiogenic properties in breast cancer cells.

Material and methods: 102 operated breast cancers were analysed in a tumor tissue array system for immunohistochemical staining of VEGF, KDR and TSP-1 allowing semi-quantitave estimatiion (0-3) of the cytoplasmatic staining intensity. In addition, VEGF was analysed by an enzyme immunoassay (ELISA) using tumor extracts prepared from frozen tissues.

Results: Cytoplasmatic staining of VEGF was observed in all samples, whereas KDR and TSP were negative in seven and twelve tumors respectively. The analysis of VEGF using ELISA correlated with the array VEGF-staining ($p=0.007$) but not with KDR- or TSP-1-staining. Interestingly, VEGF, KDR and TSP-1 correlated significantly with eachother.

Conclusion: Analysis of cytoplasmatic staning of VEGF using a tumor tissue array system seems to be a useful method for VEGF quantification in breast cancer here validated using an ELISA based method. VEGF, KDR and TSP-1 were coexpressed giving support to data that KDR is upregulated during angiogenesis and that TSP-1 has a proangiogenic role. The tumor tissue array system enables excellent opportunities of simultaneous analysis of markers engaged in angiogenesis justifying further validation using larger series of tumors.

477 Spontaneous breast duct formation *in vitro*.

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Background: 22 years ago Folkman and Haudenschild reported the first demonstration of spontaneous angiogenesis *in vitro* in which capillary endothelial cells in continuous culture formed capillary tubes. They hypothesized that extracellular matrix components (ECM) act as a mandrel around which the endothelial cells wrap. We report, for the first time, the spontaneous formation of breast duct-like structures from HBL 100 cells in continuous cell culture. These duct-like structures appear to arise in a manner analogous to capillary tubes.

Materials and Methods: HBL 100 cells were transfected with the empty pMH mammalian expression vector (Boehringer Mannheim, Indianapolis, IN). Cells containing the vector were selected by growing the cells in the presence of 700 µg/ml of G418. Cells were initially grown in McCoy's 5A medium supplemented with 10% FBS. They were gradually transitioned to serum-free medium supplemented with estradiol, insulin, prolactin, EGF and hydrocortisone.

Results: Duct formation was not observed in the presence of serum. 11 days after the cells had been passaged into serum-free medium supplemented as above, duct-like structures appeared within the confluent culture. Upon inspection at higher magnification, there appeared to be cords of a refractile material running parallel to one another around which the breast cells wrapped. These cords appeared to be a product of a subset of cells. Cells which lined-up along the cords producing cellular extensions 180 degrees apart which eventually contacted with adjacent cells of a similar phenotype.

Discussion: We report here for the first time the spontaneous formation of duct-like structures *in vitro*. We hypothesize that the breast cells produce an ECM component, very likely Collagen I or Fibronectin, which is used as a mandrel around which the cells wrap themselves to form a tube. The HBL 100 cells specifically recognize the cords of the ECM component and bind to it. This model system of duct formation provides the first *in vitro* tool to investigate: 1.) The response to changes in the hormonal and growth factor milieu as occur during puberty and pregnancy and, 2.) The effect of specific genes on duct formation.

478 The human breast stem cell model: a new paradigm in proliferative breast pathology.

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Background: In recent studies we have advanced direct evidence for the existence of phenotypically Ck5+ committed stem cells in the adult human breast epithelium that give rise to both glandular (Ck8/18+) and myoepithelial cells (smooth muscle actin = SMA+) via intermediary cells (Ck5+;Ck8/18+ or Ck5+; SMA+).

Maerial and Methods: To further test the validity of this model we investigated the whole range of benign proliferative breast disease (n=32), non-invasive and invasive breast carcinomas (n=37), and myoepithelial carcinomas (n=3) by using the same set of differentiation markers (Ck5; Ck8/18,). We applied immunofluorescence staining techniques that allow us to demonstrate simultaneously two different antigens within the same cell. The results were corroborated by biochemical studies.

Results: The data obtained provide for the first time conclusive evidence that the stem cell concept contains important insights into the nature of benign and malignant proliferative breast disease. Thus all benign proliferative breast lesions are phenotypically stem cell (Ck5+) lesions with glandular and/or myoepithelial differentiation, differing only in amounts and spatial ordering of their glandular and myoepithelial lineages. In contrast more than 90% of non-invasive and invasive breast cancers represent phenotypically a pure glandular differentiation (Ck8/18+). Only a small percentage of breast cancers constitute early glandular (Ck5+;Ck8/18+) and even more rarely early myoepithelial phenotypes (Ck5+; SMA+).

Conclusions: We conclude from these studies that BPBD (including UDH) and breast cancer are different and usually not related processes. Western blot and first pilot studies of the receptor expression of the different lesions support this view. Thus the stem cell model provides a conceptual foundation for a better understanding of both regeneration of normal breast epithelium and proliferative breast disease. Furthermore it provides a means for detailed molecular studies aimed at identifying the cell biological regulatory mechanisms of these processes. A detailed understanding will lead to better insight into the molecular mechanisms of proliferative breast disease and furthermore to novel strategies for designing new therapies.

479 Loss of heterozygosity in premalignant lesions and invasive tumors of the breast: examination of 26 commonly deleted chromosomal regions.

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Background

Despite tremendous efforts to identify genetic events involved in breast cancer etiology, many molecular dysfunctions associated with breast cancer remain unknown. The complete sequencing of the human genome has revealed that tumor suppressor genes (TSGs) often cluster in small chromosomal regions. We have taken a high-throughput genotyping approach to examine 26 chromosomal regions, including the BRCA1, BRCA2 and TP53 gene regions, frequently deleted in breast cancer tumors, to identify TSGs and TSG gene clusters involved in a variety of breast cancers.

Materials & Methods

Approximately 100 tumor samples have been studied, including cases of atypical ductal hyperplasia, in situ carcinoma and infiltrating carcinoma (stages 0 - 3). DNA was extracted from relatively homogenous cell populations obtained by laser microdissection technology. First-pass genotyping on a 96- capillary electrophoresis sequencer equipped with ultra-high throughput genotyping software involved the use of two microsatellite primer pairs per chromosomal region for development of a global LOH map.

Results & Discussion

Fine mapping was then carried out on the subset of samples showing specific chromosomal loss using large, custom panels of microsatellites. Of note, ~60% of DCIS cases show a loss of heterozygosity on chromosome 8p22 with a commonly deleted region encompassing the deleted in liver cancer 1 (DLC1) gene. Development of a genomic LOH map, representing 26 loci on 17 chromosomes, will greatly facilitate the identification of novel tumor suppressor genes and/or surrogate markers for breast cancer.

480 Screening for chromosomal alterations on chromosome 8 p11-21 in breast cancers: contribution of laser capture microdissection and tissue microarrays technology.

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Background :Alterations of chromosomal region 8p11-21 are very frequent in breast cancers but most of the genes involved have not yet been identified. To determine genomic subregions in which theses genes may be located, searches for loss of heterozygosity have yet been conducted on total tumoral samples including carcinomatous and stromal cells but never reported on pure tumoral cells.

Material and methods :Laser Capture Microdissection (LCM) was performed to obtain pure tumoral cells without surrounding normal breast in a series of 52 consecutive breast tumoral samples and then a search for LOH was conducted at 13 microsatellites markers from region 8p11-p21. Using tissue microarrays, containing 480 samples (diameter 0.6 mm each) from the tumor center and normal peritumoral tissue of paraffin-embedded breast carcinomas, we analyzed by immunohistochemistry (IHC) the expression in the same tu mors of the protein product of three potential tumoral genes lying close to or within the subregions of LOH :TACC1 (Transforming acidic coiled-coil 07-229, Upstate Biotech, Euromedex), NRG1(Neuregulin 1 aAF-296, bAF-396, R&D systems), FGFR1(Fibroblast growth factor receptor Flg C-15, Santa Cruz).

Results:two-thirds of tu mors showed LOH at at least one marker whereas no LOH was observed in the corresponding peritumoral tissues. Four subregions of LOH were identified and sharply defined compared with previous studies. By IHC on tissue microarrays, the TACC1 gene product was downregulated in tumor cells as compared to normal cells in most samples. We found a correlation (p=0.043) between the presence of LOH at 8p11-21 and a strong anti-ERBB2 IHC staining.

Discussion :Our results show that the centromeric portion of chromosome arm 8p is frequently altered in breast tumor cells and thatTACC1 is one of the potential tumor suppressor genes implicated in mammary carcinogenesis. They also suggest LCM allows to better define LOH subregions and tissue microarrays could be a powerfull tool to validate the implication of potential tumor genes.

The contribution of this technology could improve target genes identification in mammary carcinogenesis.

481 The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma.

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Purpose: LKB1 (also called STK11) is a recently identified tumor suppressor gene whose mutation can lead to Peutz-Jeghers syndrome, characterized by gastrointestinal polyps and cancers of different organ systems. Weak expression of this gene does occur at certain frequency in sporadic breast cancer. This indicates that LKB1 gene may relate to the tumorigenesis of breast cancer.

Experimental Design: To investigate the function of LKB1 gene in sporadic breast cancer, we reintroduce LKB1 into these cell lines which lack LKB1 gene. Also we examine the LKB1 protein expression in human breast cancer samples.

Results: We found that reintroducing LKB1 into these cell lines suppresses cell growth by G1 cell cycle block. The LKB1 mediated G1 cell cycle arrest is caused by up-regulation of the expression of p21WAF1/CIP1 in breast cancer MDA-MB-435 cells. We also demonstrated that the low LKB1 protein expression correlates with higher histological grade (P =0.013), larger tumor size (P =0.001), progesterone receptor status (P =0.048), and presence of lymph node metastasis (P =0.003). Furthermore, LKB1 low expression was associated with a higher relapse rate (P =0.002) and a worse overall survival (P = 0.008).

Conclusion: LKB1 plays a tumor suppressor function in human breast cancer. LKB1 expression may be a useful prognostic marker in human breast cancer.

482 BRCA1-mediated large-scale chromatin unfolding and transcriptional regulation.

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Germ line mutations in BRCA1 confer elevated risks in the development of familial breast and ovarian cancers. BRCA1 encodes a 1863-amino acid protein with a highly conserved RING finger domain at the amino terminus and two BRCA1 C-terminal (BRCT) repeats at the extreme carboxyl terminus. While the exact biochemical function(s) of the BRCA1 protein remains to be elucidated, mounting evidence points to a role of BRCA1 in regulation of multiple nuclear processes including DNA repair and transcription. The multifunctional nature of BRCA1 has raised the possibility that the polypeptide may regulate various nuclear processes via a common underlying mechanism such as chromatin remodeling. However, there lacks direct evidence in mammalian cells for BRCA1-mediated changes in either local- or large-scale chromatin structure.

We show that targeting BRCA1 to a specific chromosome location in the mammalian genome resulted in large-scale chromatin decondensation. This unfolding activity was largely conferred by the BRCT repeats. Cancer-predisposing mutations that resulted in gross truncation of the protein abolished chromatin unfolding, whereas mutations in the 3' region of the gene led to marked enhancement of the activity. A novel cofactor of BRCA1 (COBRA1) was recruited to the chromosome site by BRCA1 and was itself sufficient to induce chromatin unfolding. COBRA1 encodes a 580 amino acid protein rich in leucine residues (17%). It also contains three repeats of the LXXLL motif present in nuclear receptor coactivators and corepressors. We are currently examining the possible role of COBRA1 in regulating estrogen receptor (ER) expression.

483 The nuclear protein MRG15 is overexpressed and associated with the tumor suppressor protein Rb in human breast cancer cell lines.

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Breast cancer is characterized by uncontrolled proliferation, which must be accompanied by escape from replicative senescence. Further, it is now clear that in order to progress, cancer cells must overcome senescence in the presence of strong mitogenic stimuli, and after chemotherapy treatment. Thus, understanding the genetic mechanisms that enable a cell to bypass senescence is gaining increased relevance in breast cancer research. MRG15 is a widely conserved, largely uncharacterized member of a family of nuclear proteins related to MORF4. MORF4 induces senescence when introduced into a subset of cell lines; however, MRG15 exhibits characteristics indicative of a protein that promotes cell cycle progression and escape from senescence. MRG15 does not induce senescence, its expression levels decrease at senescence and rise during cell cycle progression, and transfected MRG15 is found to complex with and inactivate the tumor suppressor protein Rb. Together, these data suggest MRG15 plays a role promoting the cell cycle and/or evading senescence, and, therefore, its overexpression may confer a selective proliferative advantage to a cell. Based on this possibility, we examined the expression of MRG15 by western blot in 9 breast cancer cell lines and in normal human mammary epithelial cells. Compared to normal breast cells, we found that MRG15 was highly overexpressed in MDA-MB-468, BT474, MDA-MB-175, MCF-7, ZR-75 and T47D cell lines, but not in MDA-MB-231, MDA-MB-435A, or HS578t. To investigate further the role of MRG15 in breast cancer cells, we performed an Rb immunoprecipitation followed by MRG15 western blot. We detected association between Rb and MRG15 in the Rb positive cell lines T47D and MCF-7, but not in the Rb negative cell line MDA-MB-468, or HS578t, which expresses low levels of MRG15. The finding of widespread overexpression of MRG15 in breast cancer cell lines is consistent with the idea that MRG15 confers a growth advantage to the cell, and experiments testing this hypothesis are on-going. As senescence and the Rb pathway are critical for tumor response to chemotherapy, understanding the role of MRG15 overexpression in breast cancer could provide a predictive factor.

484 The LIM domain gene LMO4 is overexpressed in breast cancer and forms a complex with the tumor suppressor BRCA1.

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LIM domain proteins are characterised by the presence of one or more cysteine-rich, zinc-binding domains and have critical functions in lineage specification and differentiation in diverse cellular systems. The nuclear LIM-only subclass (LMO) of proteins comprise two tandem LIM domains that mediate protein-protein interaction. We and others have identified LMO4, the fourth member of this subclass, by virtue of its interaction with the Lim-domain binding protein Ldb1. Recently we have demonstrated that LMO4 is developmentally regulated in the mammary gland (1). Overexpression studies in a mouse mammary epithelial cell line have shown that LMO4 inhibits mammary differentiation. Significantly, LMO4 was found to be overexpressed in a high proportion of primary breast cancers. These findings imply a role for LMO4 in proliferation.

In order to gain insight into how LMO4 functions during normal mammary development and breast tumorigenesis, we searched for interacting partners using a yeast two-hybrid screen. Several candidates were isolated including a corepressor protein. We have further identified an in vivo complex involving LMO4, CtIP and the hereditary breast tumor suppressor BRCA1 (2). We describe the functional significance of this interaction in the context of breast epithelium.

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485 Distinct patterns of *BRCA1* and *BRCA2* mutations in Korean patients with early onset breast carcinoma.

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Background: Women with early onset breast cancer account for a large proportion of breast cancer patients in Korea. Although germline mutations in the *BRCA1* and *BRCA2* genes have been extensively investigated in western countries, the prevalence and characteristics of *BRCA*-associated early onset breast carcinoma in Korean population are not known at all.
Materials and Methods: Sixty women with the breast cancer by the age of 40 were studied, independently of family history of breast and ovarian cancers. Median and ranges of age were 34.5 and 18 to 40 years. Lymphocytes from peripheral blood were studied for heterozygous mutations of *BRCA1* and *BRCA2* by direct DNA sequencing method. Paraffin-embedded tissue blocks available were used for immunohistochemistry study.
Results: Eleven deleterious mutations (18.7%, 6 in *BRCA1* mutations and 5 in *BRCA2* mutations) and 7 missense mutations, genetic variant of uncertain significance, were found out of the 60 patients.No candidate for founder mutation was observed and a half of the mutations were novel, not reported in the Breast Cancer Information Core. Three patients revealed to have 2 or more than 2 mutations in their *BRCA* genes. Most of the *BRCA*-associated patients had no family history of breast or ovarian cancers. But their relatives have more total cancers than those of the patients with wild type genes. Although the expression of the Her-/neu, cyclin D1, estrogen and progesterone receptor were less common and p53 overexpression was more common in *BRCA*-associated tumors, the outcome of the patients with *BRCA* mutations was similar to that of the patients without deleterious mutations.
Conclusions: The prevalence of *BRCA1* and *BRCA2* mutations in Korean women with early onset breast cancer was highest in the outbred populations published in the literatures. However, the penetrance of the cancer seemed to be relatively low. In addition, double heterozygotes were frequently observed in this population, which implies that there may be different genetic and etiologic factors affecting transmission and penetrance of the *BRCA* in Korean patients with early onset breast cancer.

501 Internal mammary node drainage and their role in sentinel node biopsies: the initial ALMANAC experience.

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Background:The ALMANAC trial (Axillary Lymphatic Mapping Against Axillary Nodal Clearance) is an ongoing two-stage multi-centre randomized trial in the United Kingdom comparing sentinel node biopsy (SNB) with standard axillary treatment in the management of patients with clinically node-negative breast cancer. The first stage, now complete, consisted of an audited learning process where each surgeon involved in the study had to perform at least 40 SNBs, following strict guidelines set down by the trial steering group. The data presented here is from the audit stage of the trial.

Method: In each case, the sentinel node was detected using a combined technique of injecting both a radioisotope (Technetium99m) with a blue dye (Patent Blue V) peritumourally. A static lymphoscintiscan was then performed 3 hours after the injection of the radioisotope to record the lymphatic drainage site.

Results:Data was obtained from the 12 initial surgeons involved in the study who finished their 40+ audit cases within the allocated time period, producing a total of 493 patients. There were 479 sets of analysable data. The internal mammary nodes (IMN) were visualized in 41 cases (9.1%), while in the remaining 438 patients (90.9%), no IMN were seen on the scan. Of these 41 patients, only 24 had their IMN removed. However, a further 7 patients had their IMN removed that were not shown on lymphoscintiscan. These extra 7 patients had hot internal mammary nodes noted at the time of surgery. Only 4 (0.8%) patients had histologically proven positive IMN, of which 3 of these had positive concurrent axillary disease that warranted further surgery in the axilla, regardless of their IMN status. Two complications which arose from the IMN procedure were a pneumothorax and bleeding from the internal mammary artery.

Conclusions:Removal of an internal mammary node is a routine procedure with the potential for significant complications. We have shown that only a small percentage of patients (0.2%) will have a change in the staging of their breast cancer, i.e. a positive IMN with a negative axilla, by actively seeking out the internal mammary chain of nodes. It is our conclusion therefore that routine removal of the IMN chain of nodes will not significantly alter the mortality from breast cancer or the choice of therapy, although it may add to the morbidity from it.

502 The predictive value of sentinel lymph node sampling in breast cancer staging: a meta-analysis.

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Background: Lymphatic mapping with sentinel lymph node (SLN) sampling has become a widely used technology. However, reported results have varied and comparative clinical trials have not been completed. A meta-analysis was recently completed of all studies which included full axillary dissection regardless of SLN results. The primary outcomes from this analysis were presented at ASCO 2002 demonstrating a false negative rate (FNR) across the 69 eligible studies of 8.4% with 26 studies (38%) reporting rates above 10%. A significant inverse relationship was observed between the number of patients completing study and the FNR (p = .007). We report here further analysis of this study including secondary outcomes of interest.

Materials and Methods: A systematic review of the medical literature was undertaken utilizing electronic and hand searching techniques. The relationship of FNR, Posttest probability negative (PPN) and the proportion successfully mapped (PSM) to study size, proportion with positive lymph nodes, technique used and study quality was evaluated. Study quality was assessed by two blinded observers and included a description of patient characteristics, reason for study withdrawal, test performance measures, measures of variability and a description of SLN technique. Multivariate logistic regression on FNR and PPN was utilized to estimate adjusted odds ratios.

Results: Of the more than 10,000 patients in the 69 eligible studies, 8059 (77%) completed study and 7765 (74%) were successfully mapped. More than one-half of studies reported less than a 90% rate of successful mapping. Lymph node involvement ranged from 17% to 74% across studies with a mean of 40% [39.0,41.1]. SLN sensitivity for nodal involvement ranged from 71% to 100% averaging 91% [89.8, 93.1] across all trials. PPN averaged 5% [3.9, 6.0] exceeding 10% in 15% of studies. A significant linear trend was observed between the PPN and the lymph node positive rate (p=.005) averaging 9% in studies with positive nodes in >50% patients. PSM was significantly higher and the FNR significantly lower in studies utilizing radiocolloid mapping. In studies satisfying 1 or fewer quality measures, FNR and PPN averaged 14% and 10% respectively compared to 7% and 5% among those satisfying 2 or more criteria (P<.0001). Significant independent predictors of PPN included % LN positive (p=.012), Quality Score (p=.019) and PSM (p=.026).

Discussion: Reported measures of SLN test performance vary greatly depending on sample size, risk, technique and measures of study quality. This meta-analysis supports the need for a full technology assessment of SLN mapping in order to define its precise role in the management of women with early-stage breast cancer.

503 RT-PCR detection of breast cancer metastases in sentinel lymph nodes of breast cancer patients.

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Sentinel lymphadenectomy is now standard-of-care for the management of breast cancer (BrCa) patients by experienced surgeons. Current methods fail to identify many patients with metastatic disease. We hypothesize that RT-PCR analysis of sentinel lymph nodes (SLN) will more accurately detect metastases and predict recurrence than routine histological methods. We have previously identified mammaglobin (MAM) and carcinoembryonic antigen (CEA) as exceptional markers of occult disease. Here we report the results to date of RT-PCR analysis of tumor and SLN from BrCa patients. METHODS: 1100 patients have enrolled in this multicenter trial. SLN were localized by dual injection of isosulfan blue dye and 99Tc sulfur colloid. Alternate serial sections of SLN were designated for pathology (H&E ± pan-cytokeratin IHC) or RT-PCR. RT-PCR was performed on blinded specimens from 256 patients for mammaglobin (MAM) (40 cycles) and/or carcinoembryonic antigen (CEA) transcripts (32 cycles plus Southern Blot). RESULTS & CONCLUSIONS: Both MAM and CEA PCR markers were expressed in the majority of tumors, (99% (132/133) and 94% (62/66) respectively) as well as the majority of patients with histology positive SLN (94% (76/78) and 77% (40/52)). Each marker increased detection of occult metastases in patients with histology-negative nodes. Whereas MAM PCR upstaged 46% (82/177) of these patients, CEA PCR upstaged 17% (20/115) (p=0.0002) and only 10% (12 /115) had MAM+ CEA+ SLN. To date, only MAM results correlate with distant recurrences. At 3.9 yrs follow-up, 11 MAM PCR-positive patients had distant recurrences vs. 3 MAM PCR-negative patients (p=0.17 log rank). Patients with histology negative LN who are upstaged by MAM PCR have a decreased recurrence-free survival relative to patients with histology negative/PCR negative SLN (cumulative survivals = 83% vs. 98%; percent censored = 96% vs. 98%). Although these differences do not yet reach statistical significance, they support the hypothesis that MAM PCR detection of micrometastases is a useful prognostic indicator of BrCa recurrence. More accurate detection of SLN metastases may distinguish women truly at low risk for recurrence from those patients most likely to recur and to benefit from aggressive therapy.

504 Axillary lymph node dissection in women with T1 breast cancer can be omitted by identification of prognostic factors.

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Introduction: The axillary lymph node status is still the most important prognostic factor for recurrence and survival in women with invasive breast cancer (IBC), but recently almost 60% of the patients fit IBC have negative axillary lymph nodes. The primary aim of this study was to identify a subgroup of patients with a low risk of positive axillary nodes by predictive modeling of primary tumor characteristics and to find a subgroup in which the axillary lymph node dissection can be omitted.

Patients and Methods: Between April 1994 and November 2001 463 patients with primary T1-IBC who underwent complete axillary lymph node dissection were investigated retrospectively. The lymph node status was correlated with clinical and pathological factors including age of the patient, tumor size, tumor localization, grading, histology, lymph vascular invasion, estrogen- and progesterone receptor status, DNA Index, S phase fraction, Ki-67, EGFR, p53, HER-2/neu, uPA, PAI 1, bone marrow micrometastases.

Results: With univariate and multivariate analyses results 4 factors could be identified as independent prognostic indicators of axillary lymph node metastases: tumor size p<0,001, grading p<0,02, lymph vascular invasion p<0,001, Ki-67 p<0,03. T1 IBC patients with the combination of all unfavorable factors (high-risk-group) had a positive axillary lymph node status in 75,0%. In women with favorable predictive factors (low-risk-group) only 4,8% axillary lymph node metastases were found. Patients with 1-3 unfavorable factors (intermediate-risk-group) had an axillary lymph node metastases rate of 18,8% to 40,6%.

Conclusion: It is possible to predict axillary lymph node involvement by four prognostic factors of the primary tumor (tumor size, grading, lymph vascular invasion, Ki-67). Patients with all 4 favorable factors show a very low risk of positive axillary lymph node status. An axillary lymph node dissection could possibly be omitted in this subgroup. In patients of the intermediate risk group a sentinel lymph node biopsy appears adequate. Women with all unfavorable factors should undergo a conventional axillary lymph node dissection. A multicenter prospective study in Germany is now prepared to evaluate this concept using core biopsy of the IBC to identify the risk factors.

505 Intramammary lymphovascular invasion predicts axillary sentinel lymph node positivity.

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Background: In recent studies intramammary lymphovascular invasion (LVI) has been shown to correlate with non-sentinel lymph node (SLN) metastases in breast cancer patients with a positive SLN, however its value as a predictor of SLN status itself has been less studied.

Materials and Methods: A search of the Hartford Hospital Pathology file for the year 2000 identified cases of invasive breast cancer (IBC) meeting two criteria: (1) the phrase "LVI" appeared in the diagnosis and (2) SLN biopsy (Bx) was performed. Histologic slides were re-examined to determine extent of LVI positivity and a sampling of the LVI negative cases were also reviewed. Correlations of LVI (+/- and extent), SLN status, tumor size & histologic grade (HG) were performed.

Results: 152 cases were identified, 34 of which were LVI+ and 118 were LVI-. Of the LVI+ cases 23/34 underwent SLN Bx, while 96/118 LVI- cases had SLN Bx performed. When LVI was present (N=23) 17 (74%) vs 6 (26%) of cases had (+)SLN (Chi-square p<0.001). Furthermore, when LVI was extensive 100% (12/12 cases) had (+)SLN. When LVI was absent (N=96) only 32 (33%) vs 64 (67%) had (+)SLN (Chi-square p<0.0001). T-size inversely correlated with LVI and the predictive value of LVI broke down when considering T1 vs T2-4 tumors. LVI occurred in only 10/82 (12%) of T1 tumors, 6 with (+)SLN & 4 with (-)SLN. In T2-4 tumors, large size was associated with (+)SLN even without LVI (13/24 cases 53%) but when LVI was present (+)SLN occurred in 11/13 cases (85%). LVI was inversely correlated with HG but this was of limited practical use because LVI was so rare in HG-I tumors (1/16 cases; 6%). The combination of HG-III and LVI was highly predictive of (+)SLN (14/17 cases; 82%).

Conclusion: LVI is a significant predictor of sentinel lymph node status, particularly when it is extensive. The combination of LVI with HG-III or large T-size is strongly predictive of (+)SLN. The presence and quantity of LVI should always be incorporated into the pathology report.

506 Extra-axillary sentinel nodes in breast cancer: a review of 653 procedures.

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Introduction: The aim of this study was to evaluate the incidence of extra-axillary sentinel nodes (EASNs) in both palpable and non-palpable breast cancer at our institute.

Methods: From January 1997 to March 2002, 644 patients with cN0 breast cancer were studied. The first 81 patients underwent confirmatory axillary lymph node dissection. Nine patients had a bilateral tumor and 136 patients a non-palpable tumor. Preoperative lymphoscintigraphy was performed after injection of Tc-99m-nanocolloid into the tumor in a volume of 0.2 ml and a mean dose of 2.86 mCi. The sentinel node was surgically identified with the aid of patent blue dye (1.0 ml, intratumoral injection) and a gamma-ray detection probe (Neoprobe). Sentinel nodes were step-sectioned and stained with H&E and immunohistochemistry (CAM5.2). The median follow-up duration of patients without confirmatory axillary lymph node dissection after a tumor-negative sentinel node was 18 months (range 1 - 38).

Results: In 606/653 of the procedures (93%), a sentinel node was visualized during lymphoscintigraphy. The sentinel lymph node was intraoperatively identified in 629/653 (96%) and contained metastasis in 236/629 (38%). One of 396 patients who were observed after excision of a tumor-negative sentinel node developed an axillary recurrence 22 months postoperatively. A sentinel node in at least one extra-axillary basin was depicted in 191/653 (29%). Visualized internal mammary chain nodes could be harvested in 117/136 (86%) and contained metastasis in 19/117 (16%). Other visualized EASNs, such as the supraclavicular, subclavicular, interpectoral and lateral and medial intramammary nodes, were excised in 63/85 (74%). These nodes harbored metastasis in 14/63 (22%). In 16/191 (8%), a tumor-positive EASN was found, whereas the axilla was tumor-free. More EASNs were found in the presence of non-palpable tumors in comparison with palpable tumors (41% vs. 26%). This might be caused by the fact that the average non-palpable tumor is situated deeper in the breast.

Conclusion: An overall upgrading of 8% could be achieved in all visualized EASNs. If lymphoscintigraphy would have been omitted, a tumor-positive EASN would have been missed in 2.5% of all patients. Sentinel nodes outside the axilla are more commonly seen in non-palpable breast cancer.

507 Lymphocyte subset alternations in sentinel lymph nodes of patients with breast cancer.

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Background: The sentinel node (SN) is the first node on the direct lymphatic drainage pathway from a tumor, and the lymphocytes-subset analysis of SNs provides an opportunity for assessing immunological interactions between the tumor and regional-lymph nodes. The aim of this study is to examine the lymphocyte subset in SNs compared with non-SNs, in order to speculate their immunological role, especially the antigen-presenting function.

Methods: The sentinel node biopsy was performed on patients with breast cancer, using a gamma probe after injection of radioactive tin colloid, and additional non-SNs were removed (total 4 nodes in each). Half of each node was retained for histology, and the rest was placed in RPMI 1640. Freshly excised nodes were trimmed of fat and connective tissue, and teased through a mesh stainless steel wire screen. Mononuclear cells were isolated by the density gradient separation. FITC- to CD 19 (B cell), PE- to B7 (costimulating molecule; CD80/86) and CD69 (early activation marker), and CY5-conjugated monoclonal antibodies to αβ (T cell). Flowcytometric analyses were performed to compare the paired SNs and non-SNs, and the features of lymphocyte subsets in SNs were shown.

Results: 28 SNs and 48 non-SNs were examined from 19 patients (1 SN in 10 and 2 SNs in 9 pts). The ratio of B7+/B7- B cell in SNs was significantly higher than in non-SNs (5.5% vs 3.3%, p<0.005). In SNs, there was a tendency of reducing CD69+ B cells (22.1% vs 29.0%, p<0.1), but not CD69+ T cells (37.4% vs 36.0%, p=0.84). The SN with higher B7 expression was the node with the highest expression among all nodes in 9 patients, and these nodes are supposed to be the antigen-presenting nodes (APNs). The flowcytometric analysis also demonstrated a marked high B7 expression (6.4% vs 2.9%, p<0.0001) and a reduction of CD69+ B cells (19.9% vs 29.3%, p<0.05) in APNs, but not of CD69+ T cells (32.1% vs 35.9%, p=0.40).

Discussion: In SNs, there was an increase in B7+ B cells, and a reduction in activated B cells. The percentage of cells expressing CD69 in T cells was similar in paired SNs and non-SNs. This may reflect the immunological function of SNs as APNs. Studies in progress are designed to test these possibilities.

508 Recovery time following sentinel lymph node dissection versus complete axillary dissection in patients with breast cancer.

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Introduction: Sentinel lymph node dissection (SLND) is being investigated in several clinical trials as a technique for axillary staging of patients with early breast cancer. We hypothesized that patients undergoing SLND recover faster than patients who undergo complete axillary lymph node dissection (ALND).

Methods: We interviewed 112 patients who underwent SLND (n=72), ALND (n= 32), or both (SLND followed by ALND) procedures (n= 8). All patients had clinical T1 or T2 N0M0 breast cancer. Patients who had both procedures were those with a positive sentinel node, and in these patients ALND was performed as a separate procedure within 3 weeks of the SLND. All patients had lumpectomy and whole breast irradiation. Patients completed a questionnaire examining hospital stay, presence of a surgical drain, postoperative narcotic use, return to work/normal daily activity, and ipsilateral upper extremity complications.

Results: Median follow-up for all patients was twelve months (range 1-24 months). 100% of the patients in the ALND group spent at least one night in the hospital compared to 18% in the SLND group (p<0.0001). No SLND patient had a drain placed, versus 100% of ALND patients. ALND patients required a median of 9 days of postoperative oral narcotics, compared to a median of 1 day for the SLND group (p<0.0001). Return to work and normal daily activity was much slower in the ALND group (median= 19 days) than the SLND group (median= 3 days) (p<0.0001). Persistent (>4 weeks duration) ipsilateral arm complaints were common in the ALND group, consisting mostly of numbness (56%), chronic pain (9%), and transient swelling (6%). None of the SLND patients reported arm complications 4 weeks postoperatively. Complications limiting normal daily activities were rare in both groups after 4 weeks postoperatively. There was no significant difference in hospital stay (p=0.47), postoperative narcotic use (p=0.32), and return to work/normal daily activity (p=0.09) in patients who underwent both SLND and ALND versus those who underwent ALND only. Statistical analysis was performed utilizing SAS software (SAS Institute, Cary, NC).

Conclusions: Patients undergoing SLND rarely require overnight hospitalization or surgical drains, though this was the norm in the ALND group. Patients undergoing SLND used fewer oral pain medications and returned to work/normal activity much sooner than the ALND group. Ipsilateral upper extremity complications were far more common in the ALND group. There was no significant difference in recovery time parameters in patients who underwent SLND and subsequently had ALND for a positive sentinel node as compared to those who had ALND only.

509 An audit of British practice on sentinel node biopsies.

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Background:The ALMANAC trial (Axillary Lymphatic Mapping Against Axillary Nodal Clearance) is an ongoing multi-centre randomized trial in the United Kingdom comparing sentinel node biopsy (SNB) with standard axillary treatment in the management of patients with clinically node-negative breast cancer. An audited process of learning to perform SNBs, following strict guidelines laid down by the trial steering group, preceded entry into the randomized phase, which compares SNB with standard axillary treatment. The data presented here is from the audit phase of the study.

Methods:In all cases in the study, the SNB was detected using a combined method of injecting both a radioisotope (Technetium99m) with a blue dye (Patent Blue V) peritumourally was used to locate the sentinel node. A static lymphoscintiscan was then performed 3 hours after the injection of the radioisotope to record the lymphatic drainage site. During this audit phase, the sentinel node was excised prior to the patients receiving standard axillary surgery at the individual centres involved in the study, i.e. node sampling or node clearance. A total of 31 surgeons from 17 centres throughout the United Kingdom were involved in the initial phase of the study, operating on a total of 885 patients.

Results:842 sets of data were analysable from the original cohort of 885 patients.837 patients were female and 5 male. The tumour was clinically palpable in 79.6% of cases. The mean tumour size at the time of presentation was 19.6mm (Range 1-100mm). Axillary drainage was noted on lymphoscintiscan in 69.9% of cases, while internal mammary node drainage was visualized in 8.9% of cases. The sentinel node was identified in 96.1% of cases using the above combined technique. Individually, the success rates for radioisotope alone and blue dye alone were 81.5% and 77.5% respectively. There were 33 failed localizations. A total of 1727 sentinel nodes were excised, producing an average of 2.05 nodes per patient. The average time taken for the sentinel node biopsy was 18.1 mins (Range 2-90 mins). 35.5% of axillas were node positive, with a false negative rate of 6%.

Conclusions:These results compare favourably with current available data on sentinel node biopsy. It also shows that with the benefit of a formal learning process, as was initiated during the audit phase of the study, the techniques of sentinel node biopsy can be successfully transmitted to multiple centres.

510 Cost benefit and arm morbidity following sentinel node biopsy versus axillary lymph node dissection for early breast cancer patients.

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Background: Sentinel node biopsy (SNB) in breast cancer is a promising surgical technique. Axillary lymph node dissection (ALND) may be unnecessary when sentinel lymph nodes are proven histologically negative. While phase III trials to compare SNB with ALND are underway, cost benefit and arm morbidity following SNB vs. ALND should be addressed.

Patients and methods: After a feasibility study on SNB in 200 cases of operable breast cancer, we started an observational study on SNB for clinically node-negative breast cancer patients in July 1999. The purpose of this study is to evaluate cost benefit and arm morbidity between 32 cases treated with SNB followed by ALND (ALND group) and 83 cases treated with SNB alone (SNB group). After 1-year of operation, arm morbidity (arm edema, pain, numbness, sensory disturbance, and impaired shoulder mobility) was assessed subjectively. The arm circumference 10 cm above and below the elbow was also measured in all cases. Difference in arm volume between the arm on the operated and the other side was calculated by using the formula of the volume calculation of frustrum of a cone. The Fisher exact test, chi-square test and t -test were used for analysis.

Results: There were statistically significant differences in the average length of operating time and hospital stay, and average cost of hospitalization between the SNB and ALND group: 94 min vs. 139 min, 12.4 days vs. 15.3 days, and 587089 yen vs. 718498 yen. ALND group had a significantly higher rate of subjective arm edema, pain, numbness, and sensory disturbance except impaired shoulder mobility. In addition, there was a significant difference of arm volume between the arm on the operated and the other side in ALND group, but not in SNB group.

Conclusion: SNB is associated with favorable cost benefit and negligible arm morbidity compared with ALND.

511 Sentinel lymph node biopsy using technetium-99m tin colloids of different sizes.

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Background: Axillary lymph node dissection (ALND) is an essential element for predicting the prognosis and regional control of the tumor in the treatment of breast cancer. At the same time ALND is associated with the morbidity such as pain, numbness and lymphedema. Sentinel lymph node biopsy (SLNB) is a potential alternative procedure to conventional ALND in clinically node-negative breast cancer. However, optimal method for SLNB has not been fully established yet. In this study, we prepared the technetium-99m-labeled tin colloids with different sizes and compared their efficacy in SLNB.

Patients and Methods: From September 1998 to February 2002, 184 clinically node-negative breast cancer patients were enrolled in the study at Keio University Hospital. We prepared small-sized technetium-99m-labeled tin colloid (particle size: 200-400 nm in diameter). Regular-sized technetium-99m-labeled tin colloid is 400-1000 nm in diameter. In 74 patients, a SLNB was performed using regular-sized tin colloid; small-sized tin colloid was used in 110 patients. Subsequently, all of the patients were immediately followed by ALND. All dissected lymph nodes were evaluated by routine histopathological examination.

Results: The clinicopathological characteristics of the two groups were comparable. The lymphoscintigram detected SLN more frequently in the small-sized colloid group than in the regular-sized colloid group ($p<0.01$). Small-sized tin colloid was also superior to regular-sized tin colloid in the SLN identification rate (97.3% versus 86.5%; $p=0.01$). The mean value for ex vivo counts of the hottest sentinel lymph nodes of the small-sized colloid group was significantly higher than the counts of the regular-sized colloid group ($p<0.01$). There was no significant difference in the accuracy between the two groups.

Conclusion: SLNB using the small-sized tin colloid was technically feasible and provided higher detection and identification rates than the regular-sized tin colloid.

512 Simplified mapping of sentinel nodes while maintaining accuracy.

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OBJECTIVE: Sentinel node mapping is recognized as an accurate method to identify axillary metastasis. Different mapping techniques obtain similar sentinel node results suggesting an anatomic final common pathway for lymphatic drainage. We describe the use of subareolar isosulfan blue dye to identify the sentinel node and comment on the benefits of this method. METHODS: We have obtained the sentinel nodes on 146 patients over the last 53 months using isosulfan blue dye alone. Our initial experience with peritumoral injection of 28 patients had high rates of non-visualization and false negatives (each 11%). We then used the subareolar approach on the next 118 patients without massage. After five minutes, the axilla is explored. These lymphatics are carefully followed to the sentinel node(s). Complete axillary dissection followed each exploration for the first 42 patients per protocol, and in 60% of patients overall. All patients with positive sentinel nodes had axillary dissection. RESULTS: The immediate visible benefit of using the subareolar approach was the ease of identification of the initial blue lymphatic(s) due to the more direct path of blue dye to the sentinel nodes. Changing from peritumoral to subareolar injection site improved the sentinel node visualization rate from 88% to 98% ($p<0.01$). Likewise the sentinel node false negative rate decreased from 11% to 3% ($p<0.02$), and accuracy of predicting axillary status increased from 96% to 99%. The mean number of sentinel nodes found was 2.2. The subareolar approach did not interfere with either ultrasound or wire localization. There were no allergic reactions to isosulfan blue dye. CONCLUSIONS: Use of subareolar injection rather than peritumoral injection for sentinel node mapping has improved our ability to identify the sentinel node, increasing accuracy and decreasing false negatives and non- visualizing procedures. This approach saves time for the surgeon and patient, eases scheduling of procedures, and is cost effective. Radioisotope mapping of sentinel nodes uses the subdermal or subareolar approach. This report suggests that it is not as important which dye material is used during sentinel node mapping, but rather the location of the injection in the breast. Subareolar injection is consistent with the breast lymphatic physiology, simple to use, easy to schedule and therefore is the preferred route of sentinel node mapping.

513 Lymph node metastasis detection by FDG-PET and sentinel node biopsy in breast cancer patients: comparison of these different approaches. An updating.

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Background: axillary dissection (ALND) for detection of metastatic involvement is used to plan adjuvant treatments for breast cancer (BC) patients. ALND is a costly procedure associated with various side effects.80% or more of T1 patients are node negative and might avoid ALND. Recently, sentinel node (SN) biopsy has been suggested as method of reference for the evaluation of regional nodal metastases and for the decision on the need of a ALND. SN biopsy is an invasive approach, with a not negligible risk of false negative results. Conversely, Positron Emission Tomography (FDG-PET) is a non-invasive repeatable method able to evaluate all the regional nodes in BC: our PET experience on nodal involvement in BC has given interesting data of sensitivity and negative predictive value, comparable with SN biopsy. The aim in this work is a direct comparison between the two methods in term of sensibility, accuracy and predictive value in the same series.

Methods: T1N0 BC patients were studied. FDG-PET has been performed no later than 48 hours before surgery. Lymphoscintigraphy has been performed within 6 hours before surgery. After breast surgery, radio-guided biopsy of the SN has been performed followed in all cases by a complete ALND. Metastatic involvement of the SN and the other non-SN has been evaluated on definitive sections and represented the basis of the comparison between PET imaging and SN biopsy.

Results: Until now 42 patients have been studied. The average age was 55 years (range = 24-70). All patients had pT1 BC except 3 pT2 (size less than 2.5 cm). The average histological tumor size was 15 mm (range = 2-25 mm). All lymph nodes detected by lymphoscintigraphy were in axillary region, and detection rate was 100%. All SN were identified with intra-operative gamma probe, and then biopsied. All patients underwent ALND (on the average, 19 lymph nodes surgically removed). 15 patients out of 42 showed nodal metastases(35.7%); 9 out of 15 nodal involved patients had only one metastatic node. The SN biopsy results showed 3 false-negative (2 partial and 1 embolic involvement detected in non-SN), whereas FDG-PET failed to detect 4 axillary nodal involvement (2 microembolic, 1 partial and 1 pluriembolic); one patient with partial nodal involvement was undetected both the methods. One false positive FDG-PET scan was registered.

Conclusions: This is the first study comparing these two different methods on the same series. The preliminary results suggest a similar sensitivity, thus giving a contribution to a further statement on validity of FDG-PET for evaluation of BC regional node involvement.

514 A prospective comparison of positron emission tomography scanning, sentinel lymph node biopsy and axillary dissection in staging patients with early stage breast cancer.

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Introduction: Positron emission tomography (PET) using 2-fluoro-2-deoxy-D-glucose (FDG) is a noninvasive imaging modality that can demonstrate malignant lymph nodes. Axillary lymph node dissection (ALND) is the current gold standard for determining the stage and prognosis in early stage breast cancer. Sentinel lymph node biopsy (SLNB) is a less invasive procedure that can accurately stage the axilla for metastatic disease. The objective of this study is to determine the sensitivity, specificity, positive and negative predictive values of PET scanning in staging the axilla in breast cancer.

Methods: Patients with clinical stage I or II breast cancer underwent whole body FDG PET scanning 1 week prior to SLNB and simultaneous ALND, in a prospective, blinded protocol. ALNDs were evaluated by standard H & E staining, while SLNBs were also examined for micrometastatic disease using serial sectionning and immunohistochemical staining.

Results: 81 patients were recruited for the study and PET scan data were available for 74 patients. The analysis yielded the following test properties when PET scanning was compared to ALND: sensitivity 0.46 (95% CI 0.19,0.75), specificity 0.983 (0.91,1.00), PPV 0.857 (0.42,1.00) and NPV 0.891 (0.79,0.95). The positive likelihood ratio was 26.8 (3.51,203.78), and the false negative rate was 0.538 (0.25,0.81). PET scanning compared to ALND and SLNB positive by H & E yielded: sensitivity 0.41 (0.21,0.64), specificity 0.978 (0.88,1.00), PPV 0.90 (0.56,1.00), and NPV 0.78 (0.65,0.87). The positive likelihood ratio was 18.8 (2.54-139.4) and the false negative rate was 0.591 (0.36,0.79). There was one false positive PET scan. Primary tumours were demonstrated by PET scanning in only 54% of patients.

Conclusions: In this study PET scanning showed high specificity and PPV in identifying axillary metastases in early stage breast cancer; however sensitivity was low. The high false negative rates of PET scanning versus ALND and SLNB, indicate that a negative PET scan may not accurately reflect true nodal disease status.

515 Validation of different radioguided techniques for sentinel node localization in breast cancer patients.

Ponzone R, Biglia N, Elia O, Maggiorotto F, Sismondi P. Mauriziano Umberto I° Hospital and Institute for Cancer Research and Treatment (IRCC) of Cand, Turin, Italy

Background: Sentinel lymph node dissection (SLND) is increasingly accepted as standard of care for breast cancer. However, uncertainties still exist on the best way to achieve optimal results. The aim of this study is to evaluate the correspondence of the subdermal (over the tumor) injection as compared to the periareolar injection of the radiocolloid tracer in a series of consecutive breast cancer patients.

Materials and Methods: Since May 1999 to May 2002, 238 patients with invasive breast cancer T1-2 (<3 cm) N0 M0 underwent SLND. After the subdermal injection of colloid particles of human albumin marked with ^{99m}Tc, two views lymphoscintigraphic images and a hand-held gamma probe were used to locate all radioactive nodes. Axillary dissection (ALND) was performed only if histology showed a metastatic sentinel node (SLN). Since March 2002 a pilot study was started: we first injected subdermally 300 µCi of radioactive tracer, located the SLN by dynamic lymphoscintigraphy and assessed its radioactivity after 30'. We then injected peritumorally 150 µCi of tracer, verified if other SLNs appeared on lymphoscintigraphy and quantified the radioactive activity of all SLNs after 180'.

Results: At least one SLN was identified in 233/238 cases (98%). Mean number of identified SLNs was 1.7. Mean tumor size was 1.5 cm. Overall, 74/238 patients had at least one involved SLN (31%), which was the only positive node in 51/74 of the cases (69%). At least one SLN was detected in all the 20 patients of the ongoing pilot study, and the second (periareolar) injection never identified different SLN(s) as compared to the first (subdermal) injection. The migration of the tracer to the same SLN(s) with both types of injections was warranted since the intensity of the signal, expressed as the 180'/30' ratio of radioactive counts, was always > 5 with the double injection, while it was always <2 after a single injection.

Discussion: Evidence is growing that ALND is required only in patients with a positive SLN. Subdermal injection of a radioactive tracer provides optimal SLN detection, but requires previous localization of non-palpable tumors and may cause a "shine-through" effect in tumors of the upper outer quadrant. Subareolar injection of the radioactive tracer appears to provide a useful alternative for these cases.

516 Sentinel lymph node biopsy before neoadjuvant chemotherapy - conservation of breast and axilla.

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Background: Sentinel lymph node biopsy has shown to be a very accurate method of predicting the axillary status. In specially selected cases we performed SLN biopsy in patients before being treated by neoadjuvant chemotherapy on order to receive the real nodal status. We now represent the result on our first 11 patients, especially aiming at the question how many women could be treated by breast conserving surgery with SLN biopsy instead of mastectomy and axillary dissection. Methods: 11 patients with a tumour size between 2,5 and 4 cm underwent SLN biopsy before neoadjuvant chemotherapy. Lymphoscintigraphy was performed with 99mTechnetium-labelled radiocolloid. The SLN was identified using a hand-held gamma counter and examined by means of H&E and cytoceratin immunohistochemical staining. After SLN biopsy all patients received 3-4 cycles chemotherapy with epirubicin and cyclophosphamide. Surgical treatment was finished by breast conserving therapy or mastectomy and secondary axillary dissection, if necessary. Results: In one of our 11 cases no SLN could be detected (detection rate 90,9%). From the remaining 10 patients 3 reached the aim of breast conserving therapy without axillary dissection (30%). One patient had to undergo secondary mastectomy but needed no axillary clearance. Therefore 40% of our patients could be treated by SLN biopsy alone. We could perform breast conserving surgery in 80% of our patients. In case of tumour occupied SLN we found micrometastases in 2 (33,3%) and macrometastases in 4 cases (66,6%). All of the lymph nodes removed after neoadjuvant chemotherapy were free of tumour cells. Conclusion: Sentinel lymph node biopsy followed by neoadjuvant chemotherapy is a very favourable method for patients with a tumour size of 2,5 to 4 cm. 30% of these patients reached the aim of SLN biopsy and breast conserving surgery instead of mastectomy and axillary dissection. 40% needed no axillary dissection and mastectomy could be avoided in 80%. Additionally sentinel lymph node biopsy before chemotherapy predicts the axillary status more precisely than axillary dissection after chemotherapy.

517 Sentinel node in breast cancer : pathological parameters which predict the invasion of non sentinel nodes.

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Background : Sentinel node procedure is a widely used technic in breast cancer which can avoid the side effects of an axillary clearance. All patients cannot come through this procedure and can we predict which one will do safely. The aim of the study was to identify the pathological parameters associated with a positivity of the non sentinel nodes of the axilla.

Material and Methods : From our series, 401 patients have underwent the sentinel node (SLN) procedure with radiocolloid followed systematically by an axillary clearance (ALND) in order to detect in which situation the clearance is mandatory. The sentinel nodes have been processed individually in multiple slides and immunohistochemistry (IHC) was used to detect micrometastases. The rest of the axilla has been processed as usual. The pathological parameters have been correlated with the status of the axillary nodes. The status of the axilla has been separated in four groups as following : SLN-/ALND-, SLN+/ALND-, SLN-/ALND+, SLN+/ALND+.

Results : The distribution of the four groups is as following : SLN-/ALND-(59%), SLN+/ALND-(26%), SLN-/ALND+(4%), SLN+/ALND+(12%). There is a highly statistically significant ($p<0.001$) heterogeneity between these groups for several pathological parameters i.e. size of the tumor, presence of vascular invasion in/around the tumor, grade of the tumor. All these parameters are associated with the positivity of the sentinel nodes and of the non sentinel nodes, but the most discriminant parameters associated with the non sentinel node positivity is the positivity of the sentinel node by a macrometastasis (>2mm)(OR :12.8). This impact is higher if there is a capsular effraction (OR :24). When the sentinel node is invaded by few tumor cells only detected by IHC, none of the non sentinel axillary nodes from these 25 cases are invaded.

Discussion : The patients can be selected for the sentinel node procedure on basis of pathological parameters and not all patient with a positive sentinel node should undergo a complete axillary clearance.

518 The impact of immunohistochemistry (IHC) on axillary nodal positivity.

Alleyne R, Buchanan C, Holmes DR, Nakamura SK, Silberman H, Waisman JR, Woo CS, Silverstein MJ. University Southern California, Los Angeles, CA

Background: Axilllary lymph node status is important in staging and treatment of invasive breast cancer. All of our historical outcome data, however, are based on routine sectioning and staining using hematoxylin and eosin (H&E). We questioned the impact of immunohistochemistry (IHC) on axillary lymph node involvement and outcome.

Materials and Methods: From 1979 to 1996, we did 2256 level I/II axillary dissections for breast cancer (average number of nodes = 18) in which the nodes were processed in the standard fashion (node bisected and stained with H&E). More recently, we have performed sentinel lymph node biopsy in 504 patients. The sentinel lymph nodes were serially sectioned and evaluated by IHC if negative by H&E. All data were collected prospectively.

Results: Sentinel lymph node technology increased average nodal positivity in every T category (TMN Staging System). No significant difference in breast cancer specific survival was seen in patients who were upstaged by IHC, however, follow-up was too short in IHC positive patients since this is relatively new technology.

Discussion: Sentinel lymph node biopsy combined with serial sectioning and IHC increases axillary nodal positivity across all T categories by an average of 8% (range 4-12%). Further study will be required to determine whether those patients who were previously axillary node negative and who are now axillary node positive will fare worse than those who continue to be axillary node negative when their sentinel lymph node is analyzed by IHC.

T Category	% Positive by H&E	% Positive by H&E or IHC
Tis	0%	7%
T1a	5%	18%
T1b	16%	20%
T1c	29%	41%
T2	48%	60%
T3	67%	78%

519 Upstaging of sentinel nodes in breast cancer by cytokeratin 19 RT-PCR.

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Background: RT-PCR can identify single cell metastases in sentinel lymph nodes (SLN). We used RT-PCR to detect epithelial marker cytokeratin 19 (CK19) and compared use of this technique in half frozen nodes versus 5micron frozen sections.

Methods: SLNs were identified using a combination of radioactive colloid and blue dye. A level II axillary node clearance was performed in all cases. For the first 54 patients, the SLN was bivalved and half analysed by H&E paraffin histology and the other half snap frozen and stroed at -80c for subsequent RT-PCR. In the subsequent 50 patients, 5 x 54 micron frozen sections were cut from the SLN with one used for immediate H&E histology and 4 stored at -80c for RT-PCR. The remainder of each node was analysed by paraffin histology.

Results: 66 SLN were harvested from the initial 54 patients. 8 SLN (6 patients) were H&E positive. 58 SLN (48 patients) were H&E negative, however 4 patients had other axillary disease giving a false negative rate (FNR) of 40%. 24/66 SLN were CK19 positive by RT-PCR. 7/8 H&E positive and 17/58 H&E negative nodes expressed CK19. 29% of nodes (31.3% of patients) were upstaged by RT-PCR.

60 SLN were harvested from the initial 54 patients. 8 SLN (6 patients) were H&E positive. 58 SLN (48 patients) were H&E negative, however 4 patients had other axillary disease giving a false negative rate (FNR) of 40%. 24/66 SLN were CK19 positive by RT-PCR. 7/8 H&E positive and 17/58 H&E negative nodes expressed CK19. 29% of nodes (31.3% of patients) were upstaged by RT-PCR.

60 SLN were harvested fom the next 50 patients. 25 SLN (21 patients) were H&E positive. 35 SLN (29 patients) were H&E negative with 3 false negatives, giving a FNR of 12.5%. Only 15 SLN (13 patients) were CK19 positive by RT-PCR of 4 frozen sections. In 14 of these SLN the H&E frozen section had been positive. In one SLN the H&E frozen section had been negative but RT-PCR positive, however subsequent paraffin histology of this node was positive. 10 SLN that were histologically positive did not express CK19 in the 4 sections examined by RT-PCR.

Conclusions: CK19 RT-PCR upstaged 31.3% of patients where half the SLN had been examined. When RT-PCR was used to detect CK19 expression in 5micron sections, no SLNs were upstaged.

RT-PCR requires a substantial part of the SLN to avoid sampling error however this needs to be balanced with the amount of tissue examined histologically

520 GW572016, a novel dual EGFR/Her-2 small molecule, tyrosine kinase inhibitor induces regression and significant growth delay in tamoxifen-resistant, MCF-7 derived tumors.

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Her-2 upregulation appears important in the emergence of tamoxifen (TAM) resistance in hormone sensitive breast cancers. We have developed a TAM-resistant tumor xenograft (MCF-TAMR) derived from the human breast cancer line, MCF-7. In contrast to the parental MCF-7 cells, MCF-TAMR grows in the presence of TAM and has significantly increased Her-2 expression. In our study MCF-TAMR tumors were established s.c. in 27 TAM-treated NCR nude mice and tumors allowed to reach a median volume of 40 mm^3. Animals were then randomized to either placebo (vehicle) or GW572016 (100 mg/kg, oral gavage QD x 60 days) groups. Tumors were measured 3x/week and tumor volume was determined using the formula: (length x $width^2$) x ($\pi/6$). No toxicity was seen in any of the animals. There were 3 complete tumor regressions (23%) in the GW572016-treated group compared with none in the placebo arm. There was significant delay in the time to reach 5x initial tumor volume in GW572016-treated animals vs. placebo (median (days) >34 d vs. 25 d; $p=0.004$). Increase in tumor volume was significantly reduced in GW572016-treated tumors vs. placebo (4.5% median volume change vs. 9.9%; $p=0.0026$). GW572016 was seen to inhibit the activation of Her-2 and downstream survival/proliferative pathways (e.g., Erk1/2) and the expression of VEGF. These data support that Her-2 upregulation is important in the emergence of TAM-resistance. In addition, this study demonstrates that Her-2 and its related family members are ideal targets for drugs such as GW572016, which may prevent TAM resistance and offer new therapeutic choices in TAM-failure patients.

521 Synergistic combinations of EGFR tyrosine kinase inhibitors and conventional chemotherapy: in vitro median effect analysis in cell lines.

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Background: Over four hundred drugs for the treatment of malignancy are currently in development, which will overwhelm the current clinical trials process. We and others have used median effect analysis with defined cell lines to search for rational synergistic combinations of agents to advance to clinical trials. A value of CI < 1 indicates synergism, 1 additive effects, and > 1 antagonism. The current studies evaluated EGFR antagonists with classical cytotoxics.

Material and Methods: MCF7 (wild type), MCF7/adr (a multiply resistant line), and BT 474 (p53 mutant) cell lines were used. AG 1478 (an EGFR tyrosine kinase inhibitor), 5-fluorouracil, epirubicin and 5'DFUR were obtained from Sigma Aldrich, docetaxel from Aventis Pharmaceuticals and GW282974X (an inhibitor of EGFR1 and Her2/neu) from GlaxoSmithKline. The cells were tested for EGFR1 and Her/2neu expression by Western blot. Cytotoxic agents or controls were incubated for 72 hours, and then evaluated for cytotoxicity (MTT assay). The IC50 combinations were determined, and then fixed drug ratios for 8 values above and below the IC50 were evaluated for effect and analyzed by median effect. CI values within one standard deviation of 1 were considered additive. All studies were done in duplicate with 4 wells per point.

Results: MCF7 did not express either EGFR1 or Her2/neu, MCF7/adr expressed both, while BT 474 expressed Her2/neu. In MCF7 cells, docetaxel demonstrated minor synergy with AG 1478 (CI = 0.7) and not with GW282974X. In MCF7/adr cells, 5- fluorouracil, 5'-DFUR, epirubicin, docetaxel with 5-fluorouracil were synergistic with 5'DFUR having a CI = 0.2. In the BT 474 cells, 5'DFUR with an EGFR inhibitor was synergistic (CI = 0.6 ± 0.03), while 5-fluorouracil was additive (CI = 0.9 ± 0.3).

Discussion: These studies demonstrate that EGFR inhibition potentates the effects of fluoropyrimidines, especially 5'DFUR. This effect was seen with both inhibitors. As capecitabine is a prodrug of 5'DFUR and 5'DFUR is converted by thymidine phosphorylase into 5-fluorouracil in tumors, an oral combination of an EGFR inhibitor and capecitabine should be advanced to clinical trial.

522 Efficacy and dose-dependent activity of ABI-007, a cremophor-free nanoparticle paclitaxel, in first-line metastatic breast cancer: integrated results of 2 phase II trials.

Ibrahim NK, Samuels B, Page R, Guthrie T, Doval D, Patel K, Rao S, Nair M, Digumarti R, Hortobagyi GN. MD Anderson Cancer Center

ABI-007 is a novel, Cremophor-free, albumin-based nanoparticle formulation of paclitaxel in development for metastatic breast cancer (MBC) and other solid tumors that enhances intratumoral penetration of paclitaxel and overcomes many safety and convenience limitations associated with Taxol®. Entrapment of paclitaxel within the Cremophor micelle hydrophobic core in the plasma compartment is not well recognized and may have significant implications for patients receiving Taxol. In contrast, the albumin-paclitaxel nanoparticles in ABI-007 allow rapid drug transport to cells and tissues via a nanotransport mechanism, resulting in potentially lower toxicity and higher efficacy. A previous phase I study established the MTD of ABI-007 at 300mg/m^2 via 30-min IV infusion q3wk without premedication or growth-factor support. Subsequently, 2 multicenter trials evaluated the antitumor activity of ABI-007 in MBC patients: 1 was conducted at 300 and the other at 175mg/m^2. Herein, data are presented for phase II patients who received first-line ABI-007 (ie, no prior chemotherapy or adjuvant therapy only). 28 and 34 patients were evaluable for antitumor response to ABI-007 175 and 300mg/m^2, respectively. A significant (P=.001) dose response was observed. A response rate of 88% (95% CI, 77%-99%) was noted in the high-dose group compared with 50% (95% CI, 32%-69%) in the lower-dose group. There was a significant (P<.001) dose response in time to first response following ABI-007 300mg/m^2: 1.4 mo (95% CI, 1.4-1.6 mo) vs 3.4 mo (95% CI, 2.3-6.4 mo) in the 175mg/m^2 group. ABI-007 was well tolerated, with neutropenia (grade 4) 22% and 7%, neuropathy (≥ grade 3) 13% and 0%, and no hypersensitivity reactions ≥ grade 3, at 300 and 175mg/m^2, respectively. In summary, first-line ABI-007 in MBC appears very active, with an acceptable toxicity profile at a dose substantially higher than possible with Taxol. The novel biological nanotransporter mechanism of ABI-007, allowing for increased intracellular availability of paclitaxel, may account for the improved activity at both doses. ABI-007 is being investigated for the treatment of metastatic breast cancer in a multicenter, randomized, phase III trial.

523 Preclinical and clinical pharmacokinetics and safety of ABI-007, a novel, cremophor-free, protein-engineered nanotransporter of paclitaxel.

Desai N, Campbell KJ, Ellerhorst J, Ibrahim N, Soon-Shiong P. American BioScience Inc

Although Cremophor is an effective solvent for paclitaxel, it is increasingly apparent that Cremophor increases the total cost of care and is responsible for many of the serious toxicities associated with Taxol®, including anaphylactic reactions. ABI-007 is a novel, Cremophor-free, protein-engineered nanotransporter of paclitaxel that appears to offer improved pharmacokinetic (PK) properties and safety compared with Taxol. Preclinical and clinical studies have suggested that ABI-007 has potential PK and paclitaxel-releasing advantages relative to Taxol. Studies in rats have shown that ABI-007 partitions out of the vascular compartment more rapidly, with resultant lower AUC (3.7 vs 5.4mcg eq.hr/mL), lower C_{max} (4.0 vs 11.8mcg eq/mL) and longer half-life (11.4 vs 7.2hr) than Taxol. Further, the PK of ABI-007 was linear in this animal model. These preclinical findings have translated into apparent PK and safety advantages in patients with advanced solid tumors. In phase I trials conducted with ABI-007 administered weekly and q3wk, the maximum tolerated doses (MTD) of ABI-007 without premedication or growth factor support were 100mg/m^2 in heavily pre-treated patients and 300mg/m^2, respectively (Ibrahim NK, et al. *Clin Cancer Res.* 2002;8:1034-1044; Campbell KJ, et al. *Proc Am Soc Clin Oncol.* 2002;21:101a). In 23 patients receiving weekly ABI-007 starting at 80mg/m^2, the MTD in lightly pretreated patients had not been reached at 150mg/m^2/wk, whereas the MTD in heavily pretreated patients was 100mg/m^2/wk. PK studies in 16 patients in a dose-finding trial of ABI-007 given q3wk showed linearity over the clinical dose range (135-300mg/m^2), with PK parameters showing the same trends as in the preclinical studies when compared with literature data for Taxol. The PK of weekly ABI-007 is currently under investigation. In conclusion, ABI-007 is well tolerated when administered either in a weekly regimen or q3wk. The improved PK and safety profile of ABI-007 may be attributed to the unique nanotransport function of ABI-007, which may lead to improved efficacy in patients with breast cancer and other solid tumor types. This possibility is currently under investigation in a randomized, phase III trial in metastatic breast cancer.

524 Evidence of a novel transporter mechanism for a cremophor-free, protein-engineered paclitaxel (ABI-007) and in vivo antitumor activity in MX-1 human breast tumor xenograft model.

Desai N, Yao Z, Soon-Shiong P. American BioScience Inc.

ABI-007 is a novel, Cremophor-free, protein-engineered biological nanotransporter of paclitaxel. Unlike Cremophor formulations, which entrap paclitaxel in Cremophor micelles, ABI-007 facilitates rapid distribution of paclitaxel to red blood cells (RBCs) and tissue and improves the pharmacokinetics (PK) of paclitaxel. Studies were conducted in athymic nude mice implanted with subcutaneous MX-1 breast tumors. In PK studies, mice received 20mg/kg radiolabeled ABI-007 or Taxol® (T); blood, plasma, and tumor tissue were sampled over 24 hr. Comparative PK analysis demonstrated a lower plasma $AUC_{0-\infty}$ and C_{max}, longer $t½_{[BETA]}$ and higher volume of distribution for ABI-007 compared with T, indicating rapid and extensive distribution of nanoparticle paclitaxel to tissues, which may result in greater antitumor activity. Moreover, plasma/blood partitioning studies showed that ABI-007 facilitates rapid uptake of paclitaxel into RBCs, resulting in lower initial and higher sustained plasma concentrations of paclitaxel compared with T. In MX-1 tumor growth studies, mice were treated with either ABI-007 or T daily for 5 days at the maximum tolerated dose (MTD) or equitoxic dose (ETD) when tumor volume reached ~150mm^3. The MTD/ETD of ABI-007 (45mg/kg) was 3.4-fold higher than that of T (13.4mg/kg). At the MTD/ETD, ABI-007 demonstrated superior antitumor activity compared with T, resulting in significant tumor growth delay (median >89 vs 25 days with T; P<.01). Complete tumor regression was observed in 100% of animals treated with 45mg/kg ABI-007, with significantly longer response duration than that observed in T-treated animals (median, >89 vs 14 days with T; P<.01). In conclusion, the ABI-007 nanotransporter of paclitaxel has an improved PK profile compared with T, can be administered at higher doses, and has greater antitumor activity against MX-1 human breast tumor xenografts at the MTD/ETD. These preclinical data demonstrate that the novel transporter mechanism of ABI-007 translates into superior antitumor activity. ABI-007 is currently being investigated for the treatment of metastatic breast cancer in a multicenter, randomized, phase III trial.

525 Preliminary evidence of antitumor activity of ABI-007, a cremophor-free nanoparticle paclitaxel, in patients previously exposed to taxanes.

Taylor C, Ibrahim NK, Page R, Guthrie T, Rao S, Digumarti R, Desai N, Soon-Shiong P. American Pharmaceutical Partners, Los Angeles, CA

ABI-007 is a novel, Cremophor-free, albumin-based nanoparticle formulation of paclitaxel under clinical development for metastatic breast cancer (MBC) and other solid tumors. ABI-007 enhances intratumoral penetration of paclitaxel and overcomes many safety and convenience limitations of Taxol®. Entrapment of paclitaxel within Cremophor micelles in the plasma compartment has not been well recognized, and may have significant implications for patients receiving Taxol. In contrast, ABI-007 nanoparticles facilitate rapid transport of paclitaxel to cells and tissues through a nanotransport mechanism, resulting in higher cellular and tissue levels, with potentially lower toxicity and increased efficacy. Phase I studies demonstrated that the MTD for ABI-007 was 300 mg/m^2 via 30-minute IV infusion every 3 weeks or 100-150 mg/m^2 weekly without premedication or growth factor support. We present here data from four phase I and phase II studies demonstrating the efficacy of ABI-007 in patients previously exposed to taxanes. 30 patients were treated with ABI-007 at doses ranging from 175 to 375 mg/m^2 every 3 weeks or 80 to 150 mg/m^2 weekly (3 weeks on, 1 week off) via 30-minute IV infusion. The majority of patients 26 of 30 (87%) had MBC. Remaining pts included 2 (7%) with lung, 1 (3%) with ovarian, and 1 (3%) with renal cancer. 21 patients were evaluable for response. Four of 21 (19%) evaluable patients had an objective response (1 CR and 3 PRs). Nine (43%) evaluable patients had stable disease and 8 (38%) progressed. Responses to ABI-007 in patients previously exposed to taxanes could be explained by inadequate uptake of paclitaxel by tumor during their prior chemotherapy because of Cremophor entrapment. Further, the dose of Taxol is limited due to the toxicity of cremophor. In contrast, following administration of ABI-007, paclitaxel readily partitions to tissues, resulting in higher concentrations of paclitaxel in tumor. In a preclinical MX-1 tumor xenograft model, this translated into improved antitumor activity compared with Taxol. A phase II study of ABI-007 in MBC patients who previously failed taxane therapy is currently in progress.

526 Phase II safety and activity trial of two dose levels of CCI-779 in women with previously treated locally advanced or metastatic cancer–interim report.

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Background: CCI-779 is a cytostatic agent that inhibits the mTOR (mammalian Target of Rapamycin) signal transduction pathway.
Methods: Randomized, open label study with 14 sites enrolling women in Europe. CCI-779 doses of 75 mg or 250 mg were given IV weekly until disease progression. Enrollment of 109 women with previously treated advanced/metastatic breast cancer was completed in Feb.2002. All patients had previous therapy with either a taxane or an anthracycline or both. Data for the first 85 patients with a median follow-up of 84.5 days are reported here. Results: The median age was 55 years. Preliminary safety evaluation indicates that treatment emergent adverse events of NCI CTC grade 3/4 reported in >5% of patients were infection, gamma glutamyl transpeptidase increase, stomatitis, leukopenia, depression and somnolence. Dose reductions were required for 28.6% of patients in the 75mg group and 41.5% of patients in the 250mg group. The most common reason for study discontinuation (75mg, N=5/42; 250mg, N=12/43) was lethargy and/or depression. Cytostatic activity was observed in this heavily pre-treated patient population progressing after at least one cytotoxic therapy with a taxane or an anthracycline. Patients with prior anti-hormone or Her2-neu therapy, also responded. Stable disease or PR for > 3 months was observed (75 mg, N= 7; 250 mg, N= 10). Her2-neu and hormone receptor positive as well as negative patients with visceral and soft tissue metastatic disease were among the patients who responded. Biologic markers of response are under investigation. Conclusion: Preliminary data suggest activity of both doses of CCI-779 monotherapy given IV weekly to women with previously treated advanced/metastatic breast cancer.

527 Long-term administration of indomethacin in locally advanced breast cancer: clinical and pathological results of a randomized study.

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Background: Levels of prostaglandins are elevated in most malignant human tumors. NSAIDs as selective prostaglandin blockers are known to reduce risk of cancer incidence. NSAIDs may be effective also in treatment of cancer patients. Based on this assumption a randomized prospective study was designed to study the efficacy of long-term administration of Indomethacin combined with chemotherapy.
Patients and Methods: 53 pts with locally advanced breast cancer (stage III) were randomized for the study. Group I - 28 pts were treated preoperatively according to the CMFAV protocol of neoadjuvant chemotherapy and served as control and Group II - 25 pts, treated by the same chemotherapy protocol plus 150 mg PO of Indomethacin daily from the 1st day during a year.
The basic scheme of the treatment was as follow:
o 1st Day: doxorubicin - 40 mg/m2 and vincristine - 0,8 mg/m2;
o 8th Day: doxorubicin - 40 mg/m2 and methotrexate - 30 mg/m2,
o 15th Day: doxorubicin - 40 mg/m2 and 5-fluorouracil- 600 mg/m2,
o 22-th Day: doxorubicin - 40 mg/m2 and cyclophosphamide - 400 mg/m2.
Modified radical mastectomy was performed after 3-4 weeks interval following 4 courses of VAM chemotherapy. The study was performed in Cancer Research Center, Moscow, Russia.
Results: The objective response (> 50% regression) rate in group I was 40% (11/28 pts), while in group II - 76% (19/25 pts). A moderate to strong pathological response (double blind pathological evaluation was performed) was found in 22% vs 48% pts, correspondently.
Conclusion: Indomethacin in combination with neoadjuvant chemotherapy offers a better clinical response than chemotherapy alone for locally advanced breast cancer. Pathological examination confirms the clinical course.

528 An EORTC IDBBC-ECSG randomized phase II trial of two different schedules of Caelyx™ in metastatic breast cancer patients.

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Background: Caelyx™, pegylated liposomal doxorubicin, is as effective and less cardiotoxic than doxorubicin (Proc Am Soc Clin Oncol: 45a, 2002). The classical single agent schedule used in breast cancer (BC) is 50mg/m^2 every 4 weeks. A more convenient schedule (every 6 weeks) was investigated by the EORTC-IDBBC and 60mg/m^2 resulted in being the recommended dose.
Materials and Methods: Metastatic (M+) BC pts aged ≥ 65 years or who refuse or present with medical contraindications to a standard anthracycline (A)-based regimen were randomized to receive Caelyx™ either to the IDBBC (arm A) or the classical (arm B) schedule. Pts were required to have measurable lesions, an ECOG performance status (PS) ≤2 and adequate organ function. Prior adjuvant A-based chemotherapy (CT) was allowed; a maximum of one line of CT (non A-based) for M+ disease was permitted.
Results: From April 2000 until May 2002, 106 pts have been randomized (arm A=52, arm B=54). Data on pt characteristics are available for 91 pts (arm A, n=45/arm B, n=46): median age 69/70; age <65 14/15; median PS 1/1; prior CT (for M+) 15(7)/13(8); prior A 1/3. In both arms the dominant site of disease is visceral and the median number of involved sites is ≥3. The most frequently reported grade 3/4 CTC non-hematological toxicities (NHT) are: arm A (n=42), stomatitis12/1, fatigue 4/-, febrile neutropenia 3/-; arm B (n=44) stomatitis 7/1, palmar-plantar erythrodysesthesia 5/-, fatigue 4/-. Congestive heart failure is reported in 1 patient. Hematological toxicity is rarely described. Clinically important activity with both schedules has been observed and an independent response review to quantify this is planned for October 2002.
Discussion: Caelyx™ is confirmed to be a safe agent (only 2 pts experienced a G4 NHT) even in a population largely composed by elderly pts. An unexpected high incidence of mucositis is observed in arm A. The study accrual will be completed by the end of June. The final analysis will allow us to draw definitive conclusions on the activity and safety of these 2 different schedules.

529 Phase I study of pemetrexed (ALIMTA) and cyclophosphamide in patients with locally advanced or metastatic breast cancer.

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Background: Pemetrexed, a novel antifolate, inhibits thymidylate synthase, DHFR and GARFT. It has activity in several tumor types including mesothelioma, NSCLC, breast and colon cancer. Cyclophosphamide has demonstrated activity in leukemia, breast, and ovarian, cancer when given in combination chemotherapy.

Objectives: To determine maximum tolerated dose (MTD) of pemetrexed and cyclophosphamide in patients with locally advanced or metastatic breast cancer.

Methods: Infusions of pemetrexed (10 min) followed by cyclophosphamide (30 min) were given on day 1 every 21days. Folic acid and vitamin B12 were given to reduce hematologic and non-hematologic toxicity.

Results: 22 females 38-82 yrs (median 57) with PS of 0/1 were enrolled. All had received 1 to 5 prior chemotherapy regimens (median 3). Pts received 1-14 cycles (median 3) of study therapy. Of a total of 96 cycles, there were 19 delays and 2 reductions of study drug for toxicity. G3/4 neutropenia (3/22) was the main hematological toxicity. Elevated transaminases (4/22), diarrhea (1/22), F/N (1/22) and hyperbilirubinemia (1/22) were the main G3/G4 non-hematological toxicities. 1 death due to respiratory failure was not related to study drug. Responses to date: 1 CR, 4 PR, 4 SD.

Dose Level # Patients	Pemetrexed (mg/m2)	Cyclophosphamide (mg/m2)	DLT's	Response
6	400	400	G3 diarrhea	PR (1)
3	500	400		
3	500	600		PR
7	500	800	G4 febrile neutropenia	PR(2); CR(1)

Conclusion: This combination is well tolerated. Full, single agent doses of both compounds have been administered. MTD has not yet been determined. Pts are currently being recruited to Pemetrexed, 800 mg/m2, Cyclophosphamide, 600 mg/m2.

530 Symptom improvement with pemetrexed for heavily pre-treated patients with advanced breast cancer.

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Symptom palliation is an important goal of therapy for patients with advanced stage cancer, particularly for those who have previously received multiple lines of therapy. Disease-related symptoms were assessed during a phase II trial of pemetrexed (ALIMTA) 500 mg/m2 in breast cancer patients who had previously received anthracyclines, taxanes and capecitabine. Patients rated pain intensity, anorexia, fatigue, nausea and dyspnea twice before the start of therapy and prior to the start of each 21-day cycle. Pain intensity was assessed with a 100-mm visual analogue scale and the other symptoms with 4-point Likert scales. Improvement in pain intensity was defined as ≥50% decrease without an increase in investigator-rated analgesic level. Improvement in the other symptoms was defined as ≥1-point change. Improvement in each symptom needed to be sustained for ≥2 consecutive cycles. Patients were qualified for analyses of each symptom if baseline scores allowed for improvement. 96% had metastatic disease. 63% had Karnofsky Performance Status ≥90 and median age was 53. 72 patients were qualified for tumor response analysis. 5 patients achieved a tumor response and 29 patients had stable disease. Patients received a median of 3 cycles (range, 1-18). Laboratory toxicities included neutropenia, anemia, and thrombocytopenia. Clinical toxicities include nausea and vomiting, fatigue, rash, and stomatitis. Results for improved disease-related symptoms were: pain intensity 17% (6/36); anorexia 24% (8/34); fatigue 24% (14/58); nausea 32% (9/28); and dyspnea 15% (6/40). 2 patients noted improvement in all 5 symptoms and 5 patients noted improvement in 3 symptoms. Median duration of cycles of improvement were: pain intensity 3 (2-7): anorexia 3.5 (2-18); fatigue 4 (2-12); nausea 4 (2-18); and dyspnea 3 (2-8). In addition to stabilizing disease, heavily pre-treated breast cancer patients receive symptomatic benefit from therapy with pemetrexed.

531 Navelbine (N) capecitabine (C) combination (Navcap); the new first line chemotherapy regimen for metastatic breast cancer (MBC): results of phase II trial.

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Navelbine (N) and 5-fluorouracil (FU) combination has been reported to be highly effective regimen for advanced breast cancer (ABC). The oral fluoropyrimidine prodrug capecitabine (C) should be able to replace the less convenient infusional 5-FU within combination chemotherapy regimens. Navelbine-capecitabine combination (Navcap) has shown promising antitumour activity in phase I study. Based on these data we conducted a phase II trial with this combination as first line chemotherapy for metastatic breast cancer. Between April and December 2001, thirty patients (pts) were included to receive navcap regimen on 3 week schedule; N: 25 mg/m^2 on d1 & d8 and C: 825 mg/m^2 twice daily from d1 to d14. Patient characteristics were: median age 54 years [range 30-77], median PS 1 [range 0-2]; 5 pts were stage IV at diagnosis, 73.5% of pts had visceral metastasis and 73.5% had more than 2 metastatic sites. Prior adjuvant/neoadjuvant chemotherapy (67%), anthra (57%), taxanes (23%), prior hormonotherapy (57%). All patients were assessable for efficacy and toxicity; a total of 195 cycles were administered with a median of 6 cycles [range 1-10]. This combination was well tolerated, the main toxicity was WHO grade 3-4 neutropenia in 13% of pts with only one pt developed febrile neutropenia. Clinical toxicity was mild; grade 2 hand-foot syndrome was noted in 1 pt, grade 3 mucositis in 1 pt, grade 2 asthenia was recorded in 17% of pts and one pt suffered of grade 3 vomiting. Two pts had complete response (CR) and 18 pts had partial response (PR) with an overall response (OR) of 67%, and 6 pts had stable disease. This activity was recorded regardless metastatic sites (68% of OR in visceral metastasis).Our results confirm that Navcap is an effective and well-tolerated regimen as first line chemotherapy for MBC.

532 PEG liposomal doxorubicin (PLD) combined with docetaxel (D): a phase I trial in stage IV breast cancer.

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Purpose: Doxorubicin in combination with D constitutes al highly active regimen for pts. with metastatic breast cancer (MBC). Previous data suggest favourable efficacy/feasibility ratio for weekly D. compared to q 3 week treatment. PLD may more effectively penetrate tumors and has less cardiac and less other toxicities than standard doxorubicin.

The aim of this study is to assess the dose limiting toxicities (DLT) of weekly D (d1, 8 15, q 28 d) and PLD (d1, q 28 d) and to determine the maximum tolerated dose (MTD) and the recommended phase II dose of this combination.

Methods: Eligibility criteria included progressive MBC with measurable and/or evaluable lesions and adequate organ function. D was escalated if 0/3 or not more then 1/6 pts. had DLT during cycle 1. DLT was defined as: febrile neutropenia, ANC<500/µl for >7 days, platelet nadir <25.000/µl or grade 3/4 non-hematologic toxicity, adequate ANC and platelet recovery. To date, ten pts. were enrolled, with 8 evaluable for safety. Metastatic location: liver 5, lung and pleural effusion 7, other sites 2; 6 pts. had received prior chemotherapy, containing anthracyclines in 3 cases.

Results: D was escalated against fixed dose of PLD (30 mg/m^2). 3 different levels were evaluated (D 25-30-35 mg/m^2). MTD was reached at level 3. The 2 pts., treated with 35 mg D, developed delayed neutrophil recovery. Incidence of worst toxicities (NCI-CTC) per pts. was as followed: stomatits Grad II 3 pts., neutropenia III° 2 pts., vomiting III° 1 pts.

Hypersensitivity reaction (HSR) to PLD occurred in 3 patients during cycle 1, when the drug was given at a conventional infusion schedule including only grade I reactions. The problem was eliminated by rechallenge with a slower rate of infusion after antiallergic premedication.

Discussion: In combination with PLD 30 mg, q 4 wk, MTD of weekly D is 35 mg. In other phase I trials, recommended PLD dose regime were supposed to range between 30 and 35 mg, q 4 wk. Therefore our dose finding study will be continued with PLD escalation. In order to reduce the incidence of infusion reactions PLD will be administered with a modified schedule during cycle 1, as described above.

533 Enhancement of breast tumor growth inhibition (BTGI) in vitro through combination of CMF, epirubicin/ cyclophosphamide (EC), and epirubicin/paclitaxel (ET) with ibandronate (IB) or zoledronic acid (ZOL).

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Background: Antitumor effects of bisphosphonates are known. Based on the synergistic effects of ZOL and paclitaxel on breast cancer cells we investigated possible synergistic antitumor effects of various bisphosphonates as IB as well as ZOL with antineoplastic drug combinations in vitro.

Material and Methods: Breast cancer cells of 17 patients were incubated for 6 days with CMF, EC, ET and IB as well as ZOL. The percentage of BTGI compared with control cultures was determined (ATP-Sensitivity-Assay). The evaluation of BTGI was performed according to the criteria of Hunter and the area under the curve-method (AUC).

Results: Especially in the range of lower doses Level of antineoplastic drug combinations (25-6.25%) synergistic effects of ZOL on the induction of apoptosis were superior to IB: CMF 48.7% vs. 17.6%, control -20.4%; EC 65.2% vs. 35.6%, control 5% and ET 66.4% vs. 39.3%, control -3.9%. The treatment of cell cultures with ET and ZOL resulted in the highest AUC value of 18309 on average (IB 17605, control 16368); EC and ZOL gained an AUC value of 15842 (IB 15355, control 12239); CMF and ZOL showed an average AUC value of 11435 (IB 8818, control 5059).

Conclusions: The combination of bisphosphonates with antineoplastic drugs reveals impressive synergistic effects on induction of apoptosis in human breast cancer cells in vitro. Zoledronic acid was the most potent compound. Clinical studies need to be performed to prove whether this combination might ameliorate the response to antineoplastic therapy without increase of side-effects.

Supported by research grants from Roche and Novartis.

534 Preliminary results from an open-label phase IIa trial evaluating the safety and efficacy of EPO906 in patients with advanced breast cancer.

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Background: Antiproliferative agents that cause apoptosis by inducing polymerization of tubulin are among the most important class of chemotherapeutic agents in oncology. EPO906 is a newly developed microtubule stabilizer that inhibits cell growth in a broad range of human cancer cell lines *in vitro*, including those that are resistant to taxanes such as paclitaxel.

Materials and Methods: An open-label multi-center phase IIa study is in progress to evaluate the efficacy and safety of EPO906 in patients with advanced breast cancer who have failed 0-1 prior therapies for metastatic disease or have progressed while on adjuvant taxane and/or anthracycline treatment. EPO906 was administered at a dose of 2.5mg/m^2 intravenously as a 5-minute bolus infusion repeated every week for 3 weeks followed by a 1-week rest period until progressive disease (PD) or unacceptable toxicity occurred.

Results: A total of 25 patients have been enrolled so far. Of the 12 evaluable patients, a partial response by RECIST criteria was observed in 3/12 (25%), stable disease was observed in 3/12 (25%), and PD occurred in the remaining 6 patients. Overall, toxicity has been minimal. Only 1 grade 3/4 adverse event (diarrhea) has been reported to date and no dose adjustments have been required. Toxicities attributed to study drug have included diarrhea, nausea, and anorexia.

Discussion: These interim results suggest that EPO906 is well tolerated and effective in patients with advanced breast cancer. Additional accrual and response data will be presented.

535 A single dose of pegfilgrastim per chemotherapy cycle allows most patients to receive an average relative dose intensity (ARDI) ≥ 85%.

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Background: Filgrastim (Neupogen®) reduces the risk of chemotherapy-induced neutropenia and enables delivery of planned doses of chemotherapy on time. Pegfilgrastim (Neulasta™) is a sustained-duration form of filgrastim that was observed to be more effective than daily injections of filgrastim in reducing the overall incidence of febrile neutropenia (11% and 19%, respectively). A retrospective analysis was performed to investigate whether pegfilgrastim also enables the delivery of planned doses of chemotherapy on time, which may positively affect survival (Budman, 1998; Bonadonna, 1995).

Methods: The two phase 3, randomized trials of patients receiving 4 cycles of doxorubicin 60 mg/m^2 plus docetaxel 75 mg/m^2 every 3 weeks for stage II-IV breast cancer demonstrated that pegfilgrastim (6 mg fixed dose or 100 mcg/kg), given once per cycle was observed to be as safe and well-tolerated as a mean of 11 daily injections of filgrastim (5 mcg/kg). This retrospective analysis reviews the ARDI (Epelbaum, 1998) received by patients in the pivotal trials. ARDI compares the administered chemotherapy dose and timing against the planned regimen. An ARDI of 100% indicates there were no dose reductions or delays over the 4 scheduled cycles.

Results: At least 90% of patients receiving pegfilgrastim in both studies received an ARDI ≥85% (table).

	Weight-based Pegfilgrastim	trial Filgrastim	Fixed-dose Pegfilgrastim	trial Filgrastim
n	149	147	77	75
ARDI ≥85%	90%	93%	91%	88%
(95% CI)	(85%, 95%)	(89%, 97%)	(85%, 97%)	(81%, 95%)

There were no statistically significant differences in the mean ARDI or the percentage of patients with an ARDI ≥85% between the pegfilgrastim and filgrastim treatment arms in either study.

Conclusion: A single dose of pegfilgrastim was observed to be as effective as daily injections of filgrastim in delivering planned chemotherapy on time for patients receiving myelosuppressive chemotherapy.

536 A single dose of pegfilgrastim reduces the incidence of febrile neutropenia in various risk strata compared with daily filgrastim following myelosuppressive chemotherapy.

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Background: We have previously reported the comparability of once-per-chemotherapy cycle pegfilgrastim (Neulasta™), a pegylated long-acting analog of filgrastim (Neupogen®), and daily filgrastim in two phase 3 studies of breast cancer patients receiving doxorubicin and docetaxel. Factors identified putting patients at greater risk for neutropenic complications include prior chemotherapy, prior radiotherapy, poor performance status, advanced cancer, and age ≥65 years (Ozer, 2000).

Methods: We retrospectively analyzed pooled data from the 2 trials to compare incidence of febrile neutropenia (FN; ANC<0.5x10^9/L with temperature ≥38.2°C) among high-risk patient groups receiving pegfilgrastim (6 mg: n=77; 100 mcg/kg: n=150) or filgrastim (5 mcg/kg: n=225) across all chemotherapy cycles.

Results: No significant differences were observed between treatments over the range of body weights, so data were pooled. The strata-adjusted relative risk was significantly ($p<0.05$) less than 1.0 (favoring pegfilgrastim) for each risk factor; see table for incidence (n, standard error) of FN.

Risk Factor		Pegfilgrastim	Filgrastim
Age (years)	< 65	10% (200, 0.02)	18% (193, 0.03)
	≥ 65	15% (27, 0.07)	22% (32, 0.07)
Prior Chemotherapy	No	10% (191, 0.02)	17% (184, 0.03)
	Yes	14% (35, 0.06)	30% (37, 0.08)
Prior Radiotherapy	No	9% (190, 0.02)	17% (191, 0.03)
	Yes	19% (36, 0.07)	33% (30, 0.09)
ECOG ≥ 0	No	9% (164, 0.02)	19% (165, 0.03)
	Yes	15% (62, 0.05)	20% (56, 0.05)
Disease Stage	II	7% (102, 0.03)	15% (95, 0.04)
	III	9% (58, 0.04)	14% (58, 0.05)
	IV	18% (66, 0.05)	29% (68, 0.06)

Conclusion: A single dose of pegfilgrastim was observed to be more effective at reducing the FN incidence than daily injections of filgrastim in various high-risk breast cancer patient groups.

537 Predicting the risk of chemotherapy-induced neutropenia in patients with breast cancer: rationale for prospective risk model development.

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Background: Chemotherapy-induced neutropenia is frequently associated with neutropenic complications (NC) ranging from hospitalization for febrile neutropenia to treatment delays and dose reductions that may compromise treatment response and long-term outcomes. Published retrospective analyses have identified a number of factors associated with increased risk of NC during chemotherapy for breast cancer, including older age, low pretreatment blood counts, anthracycline-containing regimen, and low first-cycle ANC nadir. These studies have also demonstrated the limitations of using retrospective data (eg, frequent missing data) for building such a model.
Materials and Methods: The ANC Study Group has established a prospective registry involving more than 100 centers of patients receiving chemotherapy for different tumor types, including breast cancer. The objective is to develop a valid and reliable risk model to identify individual patients at increased risk for NC. Unconditional and conditional risk models will be built based on pretreatment patient and disease characteristics, planned and delivered chemotherapy, and cycle-by-cycle NC. Model predictive performance will be assessed using ROC analysis; model calibration by agreement between model-predicted and observed probabilities; and overall accuracy by average prediction error. The optimal scoring system will be tested in a validation sample to assess the reproducibility of the risk stratification.
Results: At this early date, 50 breast cancer patients are enrolled but not yet evaluable; it is anticipated that complete data from more than 250 breast cancer patients will be available for analysis and presentation.
Discussion: Recent large practice pattern surveys show that a substantial number of breast cancer patients receive compromised chemotherapy, due primarily to NC. A reliable tool to identify patients at increased risk for NC would be valuable to aid clinical decision-making and to avoid compromised chemotherapy by targeting appropriate supportive measures to patients at greatest risk.

538 *Pseudomonas aeruginosa* subungal infections associated with weekly taxane chemotherapy.

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Transverse superficial loss of the nail plate, Beau-Reil lines, subungal hemorrhage, dyschromia, onycholysis, and subungal abscess have all been associated with cancer chemotherapy. Nail toxicity is recognized as a relatively common side effect (incidence 30-40%) of taxane chemotherapy, and appears to be more prominent with taxanes than with other cytotoxic drugs, such as doxorubicin and 5-fluorouracil. We present here the first report, to our knowledge, of five patients with breast cancer and subungal infection either culture-confirmed or clinically consistent with *Pseudomonas aeruginosa* infection, each developing within 12-16 consecutive weeks of taxane therapy.

Patient, Treatment and Toxicity Characteristics

Age	Rx Type	Drug	Time to Toxicity	Site of Toxicity
55	Met	Paclitaxel	16 weeks	B great toes
50	Met	Paclitaxel	12 weeks	R thumb
50	Met	Docetaxel	12 weeks	L thumb, R great toe
53	Adj	Docetaxel	12 weeks	all fingernails
53	Adj	Docetaxel	12 weeks	L thumb

Each patient presented with subungal fluctuance with a yellowish-greenish, malodorous exudate, associated with erythema, edema, significant tenderness and commonly, onycholysis. All patients responded to oral ciprofloxacin therapy with resolution of the exudate within 1-2 weeks, and gradual resolution of the associated symptoms over several weeks. One additional patient who had received 28 cycles of q 21 day docetaxel therapy for metastatic breast cancer, completed 19 months previously, began concurrent weekly docetaxel and oral capecitabine and developed painful onycholysis and a subungal *Pseudomonas aeruginosa* infection at the 7th week of treatment. Nail toxicity, although common with the taxanes, generally consists of dyschromia, Beau-Reil lines, and onycholysis. Weekly exposure to taxanes, particularly without interruption, appears to be associated with an increased risk for subungal *Pseudomonas aeruginosa* infection. Careful monitoring of nail changes during weekly taxane therapy may help to avoid protracted treatment interruption by early identification of significant signs of infection.

539 Final report: epoetin alfa maintains hemoglobin levels and quality of life in breast cancer patients during conventional adjuvant chemotherapy.

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Anemia and diminished quality of life (QOL) are often associated with chemotherapy (CT) and chemoradiation (CR). A cumulative decrease in hemoglobin (Hb) has been demonstrated with repeated cycles of adjuvant chemotherapy (ADJCT) in patients (pts) with early-stage breast cancer (ESBC). A 2.0-g/dL decrease in Hb was seen following 4 cycles of ADJCT in pts with mean baseline Hb of 12.1 g/dL (Lawless et al. Blood 2000;96(11; pt 2):390b. Abstr 5447). Results from two large community-based trials including breast cancer pts with anemia (Hb ~10 g/dL) and impaired QOL at baseline showed that treatment with recombinant human erythropoietin (r-HuEPO, PROCRIT®, epoetin alfa) significantly increased Hb (by a mean of ~2 g/dL) and QOL (by a mean of >11 mm in Overall QOL on the Linear Analog Scale Assessment [LASA]) from baseline ($P<.05$) during CT (Gabrilove et al. J Clin Oncol 2001;19:2875-82) or CR (George et al. Proc Am Soc Clin Oncol 2001;20:311b. Abstr 2997). The current open-label, nonrandomized, multicenter, community-based study investigated the effects of once-weekly (QW) epoetin alfa (initial dose 40,000 U subcutaneously, with dosage adjustments up to 60,000 U QW if required) treatment on Hb and QOL in female breast cancer (Stage I-III) pts receiving ADJCT (anthracycline ± taxane) for 3 to 6 months. Eligible pts had Hb of 10-14 g/dL and were receiving standard ADJCT. Pts received epoetin alfa for up to 24 weeks. QOL was measured by LASA and the Functional Assessment in Cancer Therapy-Anemia subscale (FACT-An). In an interim analysis, 721 evaluable pts (mean age 53.5 y) had a mean baseline Hb of 12.3 ± 1.0 g/dL, comparable to the Hb level of the ESBC pts in the Lawless study (the historic control group). Pts also had minimal QOL impairment at baseline. Epoetin alfa therapy during ADJCT was associated with maintained Hb levels (mean Hb increase 0.9 ± 1.4 g/dL, $P<.05$) and improved QOL parameters (mean increase in LASA scores 4.9 mm for Energy and 4.4 mm for Activity, $P<.05$). Mean change in FACT-An (0.0 ± 28.3) was not statistically significant. Epoetin alfa therapy was well tolerated. These data suggest that QW epoetin alfa maintains or improves Hb levels and QOL in breast cancer pts undergoing ADJCT. Final data will be presented. A prospective, randomized trial is warranted to confirm these results.

540 Identifying the optimal timing of HER2/neu testing in patients with breast cancer: a Canadian cost minimization analysis.

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Background: The human epidermal growth factor receptor-2 (HER2) is amplified in 20-30% of breast cancers. HER2 gene amplification and overexpression occur early in the development of breast cancer. Recent studies have supported HER2 status as a relevant factor for medical decision making. Two validated assays available for HER2 testing are immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH). Current guidelines suggest that IHC is a reasonable first test with additional testing with FISH in inconclusive cases. The current policy in Canadian provinces is to screen for HER2 upon disease recurrence. However, screening all patients for HER2 at diagnosis is associated with some practical advantages and may even avoid future costs. In this study, a cost minimization analysis was conducted to identify the most economically attractive time point for measuring HER2 status.
Materials and Methods: A decision analysis model was developed to simulate most common economic outcomes. The model incorporated the risk of recurrence in stage I-III breast cancer, the clinical utility of IHC and FISH towards the selection of optimal anticancer therapy as well as costs for HER2 testing, tissue retrieval and adjuvant chemotherapy. Sensitivity analysis evaluating various scenarios of upfront adjuvant chemotherapy based on HER -2 status was also conducted.
Results: The findings suggested that HER2 testing at diagnosis can result in cost savings ranging from $Can10 per patient with stage I disease to $Can71 per case of stage III breast cancer. The primary factor responsible for the lower cost of HER2 testing at diagnosis was the avoidance of tissue retrieval costs. The sensitivity analysis indicated that if a negative HER2 test result were used to select less costly non-anthracycline containing adjuvant chemotherapy (i.e. CMF vs. CEF) in node positive patients, then additional savings of approximately $Can4,000 per patient would be realized.
Discussion: HER2 testing at diagnosis would be modestly cost saving to the Canadian health care system. However, substantially more costs could be saved if a negative HER2 test result were used to select less expensive non-anthracycline containing adjuvant chemotherapy in node positive patients.

541 Australian economic analysis of Xeloda (capecitabine) in combination with docetaxel for the treatment of patients with advanced breast cancer after failure of prior anthracycline-containing chemotherapy.

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Background: Xeloda (X) is an oral, tumour-activated fluoropyrimidine, with synergistic effects when used with taxanes. This effect is likely due to taxane-induced upregulation of thymidine phosphorylase in tumour cells. When used in combination with docetaxel (Taxotere; T), X increases survival compared with T monotherapy. Goal: To assess the cost-effectiveness of X 1,250mg/m2 twice daily as intermittent therapy (days 1-14) plus T 75mg/m2 every 3 weeks versus T 100mg/m2 every 3 weeks. Methods: The efficacy and safety of XT versus T for the treatment of anthracycline-pretreated advanced breast cancer were assessed in an open-label, multicentre, randomised, phase III clinical trial involving 511 patients. Pharmacoeconomic data were prospectively collected on number of infusions, drug dosage and duration, number of hospitalisations and consultations for adverse events and their treatment. Unit costs were based on published estimates for Australia. Results: The increase in overall mean (median) survival with XT versus T was 2.7 (3.0) months ($p<0.05$). Mean duration of therapy was 129 days with XT versus 98 days with T, reflecting the additional time to progression with XT (2.4 months; $p<0.001$). The number of hospitalisations for treating adverse events was 143 in the XT arm and 136 in the T arm. Despite a longer treatment duration, the lower planned docetaxel dose in the XT arm resulted in a lower cumulative dose of docetaxel in the XT arm (658mg) versus the T arm (848mg). Total costs included drug acquisition, preparation and administration, outpatient/specialist visits, and hospitalisations/ consultations for adverse events. The total cost per patient per course was $A18,581 for XT and $A18,739 for T ($A158 cost saving with XT; $A1=$US0.56 May 2002). Changes to the docetaxel dose in the monotherapy arm were assessed in a sensitivity analysis. Conclusions: XT has significant survival benefits over T alone in advanced breast cancer, and is associated with a decrease in total treatment costs. Therefore, XT is a cost-effective therapy for patients with advanced breast cancer who have failed prior anthracycline-containing chemotherapy.

542 Cost-effectiveness of letrozole vs tamoxifen as 1st line hormonal therapy for postmenopausal women with advanced breast cancer: the US perspective.

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Background The P025 study was a multinational double-blind (DB) RCT of letrozole (LET) 2.5 mg/d (n=453) vs tamoxifen (TAM) 20 mg/d (n=454) as 1st line hormonal therapy (HT) for postmenopausal women with advanced breast cancer (ABC). In the primary analysis ("core phase") LET increased TTP vs TAM. In a DB "extension phase", 53% of patients randomized to 1st line LET and 50% randomized to 1st line TAM received, at investigators' discretion, the alternative 1st line HT as 2nd line HT following progression. In a combined analysis of the core and extension phases (median follow-up 32 mos. [max 56 mos.]), survival was improved in patients randomized to 1st line LET out to 2 y. Survival curves crossed at ~3 y however reflecting better survival with LET as 2nd line HT. Survival from end of DB therapy was similar in the 2 groups.

Methods Patient-level data from the P025 trial were used to evaluate the cost-effectiveness of 2 strategies for postmenopausal women with ABC (1) LET as 1st line HT with TAM as (optional) 2nd line HT vs (2) TAM as 1st line HT with LET as (optional) 2nd line HT. Cost-effectiveness of (1) vs (2) was calculated as the ratio of the difference ([1] vs [2]) in costs of breast cancer care to the difference in life-years (LYs). LYs and costs after 1st or 2nd line HT with LET or TAM were assumed to be unaffected by strategy and not considered in the reference case. Life-table analysis was used to estimate the percentage of patients receiving 1st or 2nd line HT each mo. Health-care utilization while on 1st or 2nd line HT was assumed to be independent of HT and estimated by expert opinion. Unit costs were estimated using published sources. Nonparametric bootstrapping was used for sensitivity analyses. Costs and LYs were discounted at 3% annually.

Results Mean LYs to death or end of 1st or 2nd line HT were 1.54 and 1.29 for those randomized to 1st line LET and TAM respectively (0.25 LY saved with 1st line LET). Mean costs were $7323 and $5468 respectively ($1855 incremental costs with 1st line LET). The incremental cost per LY saved with 1st line LET vs 1st line TAM was $7410 (2.5-97.5 percentiles $6470-$14865).

Conclusion Compared with TAM, LET is highly cost-effective as 1st line HT in post-menopausal women with ABC.

543 Cost-effectiveness analysis of letrozole vs tamoxifen as 1st line hormonal therapy for advanced breast cancer in postmenopausal women: UK perspective.

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Background: The P025 study was a multinational double-blind RCT of letrozole (LET) 2.5 mg/d (n=453) vs tamoxifen (TAM) 20 mg/d (n=454) as 1st line hormonal therapy (HT) for postmenopausal women (PMW) with advanced breast cancer (ABC). In the double-blind extension phase patients could receive the alternative 1st line HT as 2nd line HT following progression, at the investigators discretion. Median follow-up was 32 mths (max. 56 mths). In a prespecified analysis, LET demonstrated a statistically significantly increased TTP for 1st line HT, and survival advantage in the 1st 2 years. The survival curves crossed at approx. 3 years reflecting the greater efficacy of LET as 2nd line HT. Survival from the point of ending 1st or 2nd line HT was similar in the 2 groups.

Methods: Patient-level data from the P025 trial were used to evaluate the cost-effectiveness of 2 alternative strategies for PMW with ABC (1) LET as 1st line HT vs (2) TAM as 1st line HT, with around 50% of patients crossing over to the alternative therapy as a 2nd line HT. Survival and costs from the point of ending 1st or 2nd line HT were assumed to be unaffected by strategy and not considered in the reference case. Life-table analysis was used to estimate the percent of patients receiving 1st or 2nd line HT each mth over the full follow-up period (56 mths). Health-care utilization was estimated by a Delphi panel of 8 oncologists; unit costs and utilities were obtained from published sources. Nonparametric bootstrapping was used for sensitivity analyses. Costs were discounted at 6%; LYs and QALYs at 1.5% (UK Treasury guidelines).

Results: The mean number of LYs (QALYs) gained per patient to the end of 1st or 2nd line HT was 1.55 (1.03) for patients randomised to 1st line LET and 1.31 (0.87) for those randomised to 1st line TAM. Patients receiving 1st line LET gained an additional 0.24 LYs and 0.16 QALYs vs those receiving 1st line TAM; incremental costs were £1178 (LET £4057, TAM £2762). The cost per additional LY gained was £5328 (2.5-97.5 percentiles £3847-£14775); the cost per additional QALY gained was £8036 (£5802-£22285).

Discussion: A strategy of LET as 1st line HT is highly cost-effective compared with other generally-accepted medical treatments.

544 Trastuzumab (Herceptin®) for treatment of HER2-positive metastatic breast cancer: a cost-effectiveness analysis.

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Background: The humanised monoclonal antibody trastuzumab (Herceptin®, H) has been shown in a phase III trial to be effective and safe in the treatment of HER2-positive metastatic breast cancer (MBC). In the EU, H plus paclitaxel (P) is indicated for first-line use in HER2 3+ MBC. The objective of this study is to estimate the cost-effectiveness of H+P compared with P monotherapy.

Methods: Outcomes, costs, and cost-effectiveness were estimated using a Markov model. Mean time to progression and overall survival were estimated from a large, multicenter, international clinical trial (Slamon, NEJM 2001) of P with or without H in women with HER2 3+ MBC. 75% of patients in P arm and 47% of patients in H+P arm were enrolled in a study of H used after disease progression. To obtain unbiased estimates of survival, propensity scoring methods were used to adjust for non-random participation in the post-progression study (Rubin, Ann Intern Med 1997). The model followed patients to 5 years, using a Gompertz function to estimate the tail of the survival curve (Mark, NEJM 1995). The currency was euros, and cost-effectiveness was calculated as the incremental cost per year of life gained, with both costs and benefits discounted at an annual fixed rate of 3%. Trial data also were used to estimate the cumulative doses of H and P, and the rate and severity of heart failure. To account for first-order uncertainty in the cost-effectiveness of H+P, we used the methods of Sonnenberg and Beck (MDM, 1994).

Results: The clinical trial analyses showed that mean time to progression increased from 3.7 months with P to 9.1 months with H+P. Mean overall survival, projected over 5 years, was 15.2 months with P and 25.0 months with H+P. Total healthcare costs for P were •16,812, compared with •41,527 for H+P. The mean incremental cost-effectiveness was estimated to be •29,300 (SD •13,800, interquartile range •24,800- •36,400) per year of life gained.

Conclusions: Treatment of women with HER2 3+ MBC using first-line H+P increases response rates and prolongs time to progression. This analysis predicts an increase in overall survival by almost 10 months, representing a cost-effective clinical improvement compared with the benefits found with many cancer treatments currently reimbursed in the EU (Earle, JCO 2000).

545 Cost-effectiveness of a novel port in one hundred consecutive cancer patients.

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The need for long-term central venous access is often critical to safety, convenience, and comfort of cancer patients. Ports have been a mainstay for this access. A novel port has been developed (PASV® by Boston Scientific) with the presumption of added efficacy by its patented anti-reflux valve allowing for a heparin free system and fewer flushes to maintain patency. This study prospectively analyzed this novel port in one hundred consecutive patients.

Informed consent and IRB approval for the study was obtained. Patients were enrolled consecutively from 11/30/99-11/09/00 and followed for the life of the port or until 12/01/01 which ever came first. A single surgeon at a single cancer center placed all of the ports. The preferred method of insertion was by cut-down of the non-dominant cephalic vein but if unsuccessful then by percutaneous subclavian vein under intravenous sedation and fluoroscopic control. The routine maintenance of a port was by infusion of 10cc of saline at the end of use or every three months, blood draws had 10cc discarded prior to collection, coagulation labs were also obtained if the port had not contained heparin.

The male to female ratio was 36% vs. 64%. Left sided placement was 76% and right sided in 24%. At the time of the report 50% of the participants were alive with their ports and 50% were deceased as a result of their cancer. The average length of study of port function was 8 months in the entire group and 11 months in those still living with a range of one month to 18 months. Throughout the study heparin was only used on 30 occasions and 20 of those were due to sluggish or poor blood return. There were no problem events reported on any infusion or blood draws in 79% of the ports during the study. The ability to infuse but difficult blood draw on one or two episodes was experienced by 15% of the ports, greater than two episodes was seen in only 6% of the ports during the study. One port was removed at three months due to persistent bacteremia. There were no instances of port catheter fracture, rotation, malfunction, sepsis or the need for lytic agents.

The safely and effacacy of this catheter has been proven with the estimated total cost savings in this study by avoiding heparin, routine flushes every three months rather than monthly and the ability to avoid a second peripheral blood draw for coagulation studies was $200.00 per patient per year.

546 Cost and burden of illness associated with metastatic breast cancer.

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Background: Metastatic breast cancer (MBC) has significant burden of illness in terms of incidence, prevalence, mortality and morbidity with limited information on direct costs. Direct costs for MBC include anti cancer drugs, hospital care, physicians' services, nursing home service. The purpose of this study was to assess the burden of illness of MBC over a specific period of time as observed in Medicare program expenditure data.

Methods: Incident cases of MBC from 1997-1999 were included. Direct care cost data were obtained from Medicare claims from a 5% national sample of program beneficiaries. ICD-9 codes were used to identify MBC patients. Costs to the health care system were determined from the date of first diagnosis of MBC to the loss of follow-up. A matched case-control study provided comparison of Medicare payments in cancer and noncancer patients.

Results: 397 MBC patients were identified and followed for an average of 16.2 months. A 3:1 match with a control group was performed based on age, ethnicity, reason for Medicare entitlement, months of eligibility pre and post index date, urban or rural county and state of residence. Index date refers to the first available diagnosis of MBC patients. The control population was assigned the index date of their matched cases. Median age of the MBC cohort was 73 years. 67.5 % of the MBC patients died, 30.7% were alive and 1.8% patients were lost at the end of follow-up. The follow-up period averaged 16.2 months, with a mean cost of $35,164 per MBC patient and of $4,176 per person for the control group. Over the follow-up period, the MBC patients averaged 1.7 inpatient admissions per patient and 14.4 inpatient days per admission. The control group averaged 0.3 inpatient admissions per patient and 1.6 inpatient days per admission. Inpatient costs as a proportion of total costs varied across the age brackets of the MBC cohort. The proportional inpatient costs were highest in patients aged ≤65 years and lowest in patients ≥85 years.**Discussion**: Medicare expenditure per person for MBC patients was higher than the control group with greater inpatient admissions and length of hospitalization days. The direct costs for the older MBC patients were less than the direct costs of the younger MBC group. Therefore, these data suggest that new MBC therapies that reduce hospitalization incidence and/or length of stay may have economic benefit especially in younger patients.

547 Cost effectiveness of ductal lavage: a breast cancer risk assessment technique.

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Background: Tamoxifen has been shown to reduce the incidence of breast cancer in high-risk women. While tamoxifen appears to be cost effective as a prevention strategy, the majority of women do not choose to use it. The technique of ductal lavage, a minimally invasive and safe method of obtaining cells that line the breast ducts, is an option to identify women at greater risk for breast cancer and benefit from chemoprevention, but at additional expense.

Objective: To estimate cost effectiveness of the ductal lavage procedure to identify women at increased risk for breast cancer as compared to screening alone or tamoxifen use for five years.

Methods: A Markov model was developed to compare the following three strategies for a cohort of high-risk women defined by a five year Gail risk of 1.67 or greater: (1) attempt ductal lavage for high risk women and use of tamoxifen only by women with presence of atypia, (2) routine screening with mammography and (3) tamoxifen therapy for some proportion of the high-risk women (reference case assumed 25% of high risk women take tamoxifen). Main data sources include Breast Cancer Prevention Trial, Group Health Cooperative of Puget Sound, and the National Center for Health Statistics.

Results: The ductal lavage strategy extended expected survival by 88, 46 and 14 days for 40, 50 and 60-year-old women respectively. The tamoxifen strategy was also effective, extending expected survival by 23, 14, and 9 days for the respective age groups. Both the lavage and tamoxifen strategies were predicted to be well within the range of cost effective interventions (less than $50,000/life year saved for all ages) with the tamoxifen strategy being the most costly. The results of the model were highly sensitive to the rate of breast cancer for women with atypia on lavage, the proportion of women choosing to take tamoxifen therapy, and the costs of the lavage procedure and the yearly cost of tamoxifen.

Conclusions: Ductal lavage appears to be cost-effective in a cohort of high-risk women, although more so in younger women than older. Its effectiveness increases as the proportion of the population willing to take tamoxifen (without use of lavage) decreases, if atypia identifies a group of women at increased risk for breast cancer and with increased benefit from tamoxifen and of women choose tamoxifen based on lavage results. Therefore lavage has value if it alters patient decision-making with respect to chemoprevention.

548 Does hormone therapy for the treatment of breast cancer have a detrimental effect on memory and cognition?

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Background There is evidence from animal studies and research on patients with Alzheimer's disease and women taking HRT during the menopause, that oestrogens have an important role in cognition. The impact of anti-oestrogen therapy in breast cancer on cognitive functioning is unclear. In this pilot study the effect of hormone therapy in early breast cancer on cognition was examined.

Patients and Methods: Post-menopausal women participating in a randomised trial of anastrozole, tamoxifen alone or combined (ATAC) (n=94) and a control group of women without breast cancer (n=35) completed a battery of neuropsychological measures. The groups did not differ significantly by age or estimated IQ.

Results: Compared with women in the control group, the patients were impaired on the processing speed task (p=0.049) and on a measure of immediate verbal memory (p=0. 034). When differences in age and hormone replacement therapy (HRT) use were taken into account, the deficit in verbal memory remained significant (p=0.045). Patient group performance was not significantly related to other factors including length of treatment, extent of surgery, anxiety or depression.

Discussion This pilot study found a specific impairment in verbal memory in women receiving hormonal therapy for the treatment of breast cancer. Verbal memory may be especially sensitive to changes in oestrogen levels, a finding commonly reported in studies of hormone replacement therapy in healthy women. In view of the increased use of hormone therapies in both the adjuvant and preventative settings their impact on cognitive functioning should be investigated more thoroughly.

549 Fatigue, menopausal symptoms and cognitive dysfunction associated with adjuvant chemotherapy: first year results of a large prospective controlled study.

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Background: Fatigue, menopausal symptoms and cognitive dysfunction are important side effects in women receiving adjuvant chemotherapy. The goals of this study are to determine the incidence, severity and time-course of these symptoms, and their interrelationship and effects on quality-of-life (QL). **Methods:** Patients ≤60 years receiving adjuvant chemotherapy were invited to participate and to nominate a relative, friend or neighbor, matched by age, as a control. Patients who had received ≥3 courses and controls completed the FACT-G QL scale with sub-scales for endocrine function (FACT-ES) and fatigue (FACT-F), and the High-Sensitivity Cognitive Screen (HSCS). Evaluations are repeated 1 and 2 years later. The primary comparisons were of fatigue, menopausal symptoms and cognitive function of patients and controls. **Results:** The planned sample of 100 pairs of patients and controls has been enrolled with first year results analyzed for 93 pairs. Patients and controls were well matched for age (median 49yrs), education level and menopausal status at start of chemotherapy (55% pre-menopausal). Most patients (66%) received CEF chemotherapy. Patients experienced much more fatigue than controls (median FACT-F scores 31 vs 46, inter-quartile ranges: 22-38 vs 41-49, $p<0.0001$). Patients had more severe menopausal symptoms than controls (median FACT-ES scores 58 vs 64, $p<0.0001$), and had lower serum estradiol levels. Global QL was poorer for patients (median FACT-G scores 76 vs 93, $p<0.0001$). Fatigue and menopausal symptoms were strongly correlated ($p<0.0001$) and associated with poorer global QL. Moderate/severe cognitive dysfunction was observed in 16% of patients and 4% of controls ($p=0.001$ for global distribution), and was independent of fatigue and menopausal symptoms. **Conclusions:** Fatigue and menopausal symptoms are common, interrelated problems in patients receiving chemotherapy, and are associated with poor QL. Cognitive dysfunction is an important side effect in a minority of patients.

Dr Tannock thanks the Susan G Komen Foundation for the 2001 Professor of Survivorship award, which provided partial support to this study.

550 Impact of epoetin alfa on cognitive function, asthenia, and quality of life in women with breast cancer receiving adjuvant or neoadjuvant chemotherapy: analysis of 6-month follow-up data.

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Impaired cognition and asthenia are adverse effects associated with adjuvant breast cancer (BC) chemotherapy (CT). This pilot trial evaluated the effects of epoetin alfa (EPO) on cognitive function, asthenia and quality of life (QOL) in stage I-III BC patients (pts) with baseline hemoglobin (Hb) ≥9 g/dL and ≤14 g/dL receiving anthracycline-based adjuvant or neoadjuvant CT by comparing measurements taken at baseline, prior to cycle 4 CT, and 6 months after completion of CT. Pts were randomized to EPO 40,000 U SC once weekly (QW) or placebo (PBO) while receiving 4 cycles of CT over 3 months. Cognitive function was assessed by Executive Interview (EXIT25), asthenia by the Functional Assessment of Cancer Therapy-Anemia (FACT-An) subscale, and QOL by the Linear Analog Scale Assessment (LASA). This is a final analysis of 44 EPO pts and 39 PBO pts during CT and at 6-month follow-up. Fatigue and QOL deteriorated to a lesser extent in the EPO arm than the PBO arm during CT.

	Baseline		**Mean change (prior to cycle 4 CT)**		**Mean change (6 months post CT)**	
	epoetin alfa (SD)	PBO (SD)	epoetin alfa (SD)	PBO (SD)	epoetin alfa (SD)	PBO (SD)
EXIT 25 (% with ≥1 SD deterioration)	N/A	N/A	4.5	13.6	7.3	2.8
EXIT 25 (% with ≥1 SD improvement)	N/A	N/A	18.2	2.3	24.4	22.2
EXIT 25 (mean change)*	6.0 (3.2)	5.8 (3.1)	-1.3 (3.3)	0.3 (2.4)	-1.5 (2.9)	-1.4 (2.6)
FACT-An	62.2 (12.0)	63.3 (11.3)	-2.5 (11.9)	-7.0 (13.8)	0.6 (13.4)	-2.5 (10.2)
LASA Energy (mm)	60.7 (18.5)	64.2 (19.4)	-3.2 (25.4)	-12.8 (32.7)	2.9 (26.2)	3.7 (26.8)
LASA Activity (mm)	65.8 (20.3)	70.2 (19.9)	-0.4 (26.1)	-7.9 (33.6)	7.5 (22.2)	3.7 (24.0)
LASA Overall QOL(mm)	69.2 (22.10)	72.9 (19.1)	-3.6 (28.6)	-8.2 (24.6)	6.6 (29.0)	3.8 (24.0)
Hb (g/dL)	12.8 (0.95)	13.0 (1.0)	0.8 (1.2)	-2.1 (1.4)	Not collected	Not collected

* Decrease in score = improvement in cognition

For EXIT25, more EPO pts showed a ≥1 standard deviation (SD) improvement in cognitive function than PBO pts during CT and at follow-up. PBO pts demonstrated overall deterioration in cognitive function. At follow-up, both groups showed a restoration of cognitive function and QOL, although improvements were generally greater in EPO recipients. These results indicate the need for a larger controlled trial.

551 Correlation between fatigue and musculoskeletal symptoms and chemotherapy-induced release of inflammatory cytokines in the blood.

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Chronic fatigue during chemotherapy is often associated with anemia, however transient severe fatigue and flu-like symptoms are frequently seen within days after administration of cytotoxic therapy. We hypothesize that these symptoms may be due to transient release of inflammatory cytokines in response to therapy. The objective of this study was to correlate plasma levels of IL1, IL6, IL8, IL-10, IL12, and TNF with paclitaxel (P)- and FAC-induced fatigue and musculoskeletal symptoms during one course. Symptoms were assessed on day 0, day 3, and on the day of the next treatment using the Brief Fatigue Inventory, Hospital Anxiety and Depression Scale, and a linear analog symptom score. Patients also completed a daily toxicity inventory. Cytokines were measured at corresponding time points. Patients receiving adjuvant chemotherapy for stage I-III breast cancer were eligible. 105 subjects participated in the study, 20 patients received FAC and 70 received paqclitaxel, 15 healthy volunteers were also included as controls for the cytokine measurements. Repeated measures analysis of the quality of life data revealed that fatigue, nausea and numbness were significantly more severe in the FAC treated group compared to P. Muscle aches and flu-like symptoms also tended to be more severe but this has not reached statistical significance. There was no difference between the toxicity of weekly (n=35 and 3-weekly (n=35) schedules of P measured over one treatment course. Fatigue, nausea, numbness, and flu-like symptoms were significantly worse on day 3 than at baseline or on the first day of the next cycle. Anxiety level was highest on the first day and steadily declined during the treatment. Cytokine measurements are ongoing and results will be presented at the meeting. This study represents the first attempt to measure changes in the levels of inflammatory cytokines in the blood in response to standard dose chemotherapy

552 Comparison of psychosocial status in patients with breast cancer and patients with other oncology diagnoses.

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Background: Numerous studies have examined quality of life of patients with breast cancer. However, to our knowledge, no study has compared mental status and health status of patients with breast cancer and other oncology diagnoses. The main objective of this study was to examine perceived health status, mental health status and functional limitations in patients with breast cancer and patients with other oncology diagnoses, using the 1996 Medical Expenditure Panel Survey (MEPS) data.

Material and Methods: Data from the Household Component of MEPS, a nationally representative sample of the United States population were analyzed. Two mutually exclusive groups of female subjects, aged between 19 and 100, with the following oncology diagnoses were identified through International Classification of Diseases, 9th revision (ICD-9) codes [breast cancer (ICD-9=174) and other oncology diagnoses (ICD-9=140-173, 175-239)]. Mental health status and perceived health status were measured on a 5-point scale, with 1 being "excellent" and 5 being "poor". Descriptive statistics and analysis of covariance adjusting for age, race, and the number of co-morbid conditions were conducted. Results were weighted to reflect population estimates.

Results: 460,000 patients with breast cancer and 6.5 million subjects with other oncology diagnoses were identified from the database. Subjects with breast cancer had worse adjusted mental health status and perceived adjusted health status ratings (2.2 and 2.9 respectively), as compared to patients with other types of cancer who reported a ranking of 2.0 (mental health) and 2.6 (reported health status). 32 % of patients with breast cancer required assistance with Instrumental Activities of Daily Living (IADL), as compared to 8 % of patients with other oncology diagnoses. **Discussion:** The results of this study suggest that breast cancer might impact patients' health status and functional ability to a greater extent than other types of cancer. Having breast cancer might affect patients perception of themselves and their self-esteem, impacting their mental health. More studies are needed to assess the impact of breast cancer and its treatment on mental health status, quality of life, and functional ability of breast cancer patients.

553 A pilot study comparing the psychosocial needs of Hispanic families of breast cancer patients and survivors.

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Purpose: Little attention has been focused on the families of Hispanic women diagnosed with breast cancer. This pilot study assessed the psychosocial impact of breast cancer on Hispanic families by comparing changes in family psychosocial status over time after diagnosis.

Methods: Fifty individuals were interviewed (n=50): 25 family members of current breast cancer patients (PF) (interview within a year of diagnosis), and 25 family members of survivors (SF) (interview more than a year after diagnosis). Validated scales included the Impact of Events Scale, the Depressions Scale, the Functional Assessment of Cancer Therapy Scale, thoughts about cancer, and family communication.

Results: Both PF and SF had minimal levels of distress. Neither group as a whole suffered from depression, but gender differences were found. While male PF and female PF scored similarly, female SF scored higher than male SF, suggesting that female family members are more likely to have feelings of depression, which last over longer periods of time. PF and SF were able to maintain healthy social/family relationships, and emotional and functional well being. Both PF and SF ranked these 3 domains as important aspects of quality of life. Although both groups felt they had a good relationship with the doctor treating their afflicted family member, neither felt that this had an impact on their quality of life. Both groups showed low levels of cancer risk communication with other family members, with the most communication occurring with their mother, sisters, children, spouse, brothers, and aunts, in that order. PF were significantly more likely to have thoughts about their own cancer risk (p=0.057), and these thoughts were likely to affect their mood (p=0.011).

Discussion: The greatest impact on family members of Hispanic breast cancer patients is an increased sense of vulnerability to cancer that dissipates over time. These study results suggest that there are few changes in the quality of life of family members of breast cancer patients over time after diagnosis. However, these results need be duplicated among a larger study sample.

554 Perception of body image in women at increased risk of breast and/or ovarian cancer due to a genetic predisposition, who opt for prophylactic surgery.

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Background: Genetic testing enables women at risk for hereditary breast and/or ovarian cancer to determine whether they have inherited a pathogenic mutation in BRCA1 or BRCA2 genes, and if so, to opt for frequent surveillance or prophylactic surgery (PS) (bilateral mastectomy (PM) including reconstruction and/or oophorectomy (PO)). Little is known about the psychosocial effects of PS in high-risk women. In the PREVOM study we investigate prospectively the outcome of PS in women with a genetic predisposition. We describe the effect on level of distress and body image in 52 high-risk women who opted for PM, PO and PM+PO.

Material and Methods: Four to two weeks before surgery, and 6 and 12 months after surgery, participants filled out several questionnaires, including the Impact of Events Scale and Body Image Scale. Distress as measured by the IES and body image up to six months after PS were analysed separately for women with or without previous BC undergoing either PM, PO or PM+PO, using Multivariate Analysis of Variance.

Results: At six months after PS, thoughts on intrusion and avoidance decrease significantly in our 6 months follow up (resp. p=.009 and p=.044). However, satisfaction with the appearance of their breasts decreases in all women (p=.000), especially women who opt for PM+PO (p=.003).

Discussion: Distress significantly decreases at six months follow-up. On the basis of data from simultaneous in-depths interviews we attribute this to the relief after risk reduction as a result of PS. However, according to our results, most women are less satisfied with the appearance of their breasts after PS. In interviews, women who had undergone PM report that they perceive their breasts as smaller and feeling 'different'. Increase in weight as a consequence of menopause after PO could be related to less satisfaction with their bodies.

555 Novel curriculum for short-term psychoeducational group therapy program for newly diagnosed breast cancer patients.

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Background:28% (n=59) of 270 breast cancer patients treated at WFUBMC in an 18 month period participated in short-term group interventions. Little attention has been given to the peri-operative treatment phase.Our program offered women this service in the peri-operative phase of treatment when the trauma of the diagnosis results in significant psychological ramifications. This program was designed to address the immediate as well as extended physical, cognitive, emotional, and spiritual consequences created by the diagnosis of breast cancer.

Methods: Group sessions were 90 minutes, one time per week for 8 consecutive weeks. No more than10 women participated per group. Groups consisted of 45 minutes of didactic instruction and 45 minutes of group support. Surveys were completed at session 1and 8 assessing level of emotional distress, sense of control, and confidence level in participating as an active member of their treatment team. Ratings were based on a Lichert-type scale of 1-10 (1 = minimal and 10 = severe). Patients rated their group experience as negative, neutral, or positive.

Results:Participating women were Caucasian (94%); mean age was 46; 85% (n=50) were married, 12% (n=7) were divorced. Education level was: 79% (n=47) college degrees, 17% (n=10) high school, and 4% (n=2)<high school. The mean rating for emotional distress decreased from 7.1 to 3.4. The mean ratings for confidence in communicating with the treatment team and sense of control increased from 5.9 to 8.4 and 4.3 to 5.8, respectively. 99% (n=58) rated their experience as positive.

Discussion: This curriculum significantly decreases emotional distress in breast cancer patients. Post- group ratings suggest mild improvements in perceived sense of control and in confidence level in communicating with the treatment team. Feedback gathered from minority women who did participate in our group served to define innovative recruitment procedures that may be beneficial in recruiting for other services and clinical trials. Despite its limitations, the results of the evaluation of our curriculum are promising and warrant a clinical trial employing a larger sample size with a comparison group.

556 Patient reported side effects after 2 to 3 years adjuvant tamoxifen.

Fallowfield LJ, Murray RA, Coster S, Coombes C, Gibson L, Bliss J, Hall E, on Behalf of the Intergroup Exemestane Study Steering Committee. University of Sussex, Brighton, East Sussex, United Kingdom; Imperial College of Science, Technology and Medicine, Charing Cross Campus, London, United Kingdom; Unit, Institute of Cancer Research, Sutton, Surrey, United Kingdom

Background: The benefits of hormone therapy in prevention (Fisher et al., 1998), adjuvant (Baum et al., 2001) and advanced breast cancer (Osborne, 1998), are well known. Hormone therapy is viewed by many as less toxic than many other cancer treatments and side effects described as minimal or well-tolerated (Fellowes et al., 2001). There is some evidence that menopausal symptoms reach a peak by 12 weeks of treatment and plateau out thereafter (Fallowfield et al., 1999). The Intergroup Exemestane Study provides an opportunity to examine the patient reported side effects of women who have been taking tamoxifen for 2 to 3 years.

Method: In this multi-centre, international study, postmenopausal women with primary breast cancer treated surgically and with adjuvant tamoxifen for 2 to 3 years are then randomised to either continuation of tamoxifen or to exemestane for a further 2 to 3 years (a total of 5 years endocrine treatment). In the patients recruited to the Quality of Life (QOL) sub protocol, QOL is assessd using the FACT-ES questionnaire (Fallowfield et al., 1999). To date, 538 women (mean age 63.5 years) have completed the pre-randomisation questionnaire permitting assessment of side effects after 2 to 3 years of tamoxifen.

Results: The FACT-ES has 18 endocrine related items graded on a 5-point scale. Eight symptoms were "quite a bit" or "very much" of a problem for >10% of patients; most problematic were: hot flushes (38.5%), night sweats (27.5%), vaginal dryness (15.5%), loss of interest in sex (22.1%), weight gain (34%), bloatedness (13.4%), breast sensitivity (12.4%) and mood swings (10.7%).

Discussion: Comprehensive evaluation of patient reported side effects of hormone therapy is vital to help determine supportive interventions needed to accompany the most efficacious treatments and to aid decision making.

557 Feasibility and responsiveness of utility measurement in breast cancer patients: a prospective study.

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Introduction: Utility tools measure health-related quality of life (HRQL) over time, based upon quantification of preferences for different health states. They can also be used in economic evaluations of interventions and in decision-making analysis. We report the novel application of a utility tool, the Health Utilities Index 3 (HUI3), in early stage breast cancer (BC). Feasibility and responsiveness are assessed.

Methods: This prospective, longitudinal study assessed the accuracy of preoperative positron emission tomography (PET) scanning in staging of clinical stage I and II BC, compared to axillary dissection. The HUI3 and Short Form 36 (SF-36) were administered at enrolment (E), 24 hours after preoperative PET scanning (PP), 1 week (PS), 3 months (3m) and 6 months (6m) after either breast conservation surgery (BCS) or modified radical mastectomy (MRM). Repeated measures ANOVA was used to test differences in scores, $p<0.05$ was significant.

Results: Data for 64 women are presented. Three patients refused to complete the interviews. Mean, SD age was 55.5, 10.1 years, 66.7% postmenopausal, 93% underwent BCS and 7% MRM, and 31% were node positive. Mean HUI3 multi-attribute scores were: (E, PP, PS, 3m, 6m) = (0.74, 0.77, 0.48, 0.71, 0.69), ($p<0.01$). Changes were seen in several attribute subscales (both physical and mental) over time from initial consultation to 6 months after surgery. There was a similar pattern of results in the physical component for the SF-36 ($p<0.01$). The HUI3 took approximately 5 minutes to administer.

Conclusion: The HUI3 is both a feasible and responsive tool for measuring HRQL in the perioperative period in the surgical management of early stage BC.

558 Functional interaction between PML and Brca1 in double strand break DNA repair.

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The tumor suppressor PML is a multifunctional protein involved in the regulation of cell growth and apoptosis. Recent studies demonstrated that PML may also play a role in maintaning genome stability, cellular senescence, ALT telomere lengthening, and DNA repair. Our study here demonstrated that stable and inducible overexpression of PML significantly increased cell survival against ionizing radiation (IR). Two approaches were carried out to investigate whether increased cell survival is a result of increasing double strand break (DSB) DNA repair. (1) We perform BrDU incorporation into single stranded DNA and examine the formation of IR induced foci (IRIF); (2) We examine the reactivation of damaged plamid DNA by transient transfection and luciferase reporter assay. From these studies we concluded that overexpression of PML significantly enhanced DSB-DNA repair. To examine whether PML nuclear bodies (NBs) is the site of DNA repair, we investigated if IRIF colocalizes with the PML NB in cells exposed to IR. Our study showed that PML NBs are colocalized with the IR induced DNA repair foci. We next perform double color immunofluorescence staining using a modified procedure to investigate whether PML is colocalized with the enzymes involved in DSB-DNA repairs. Result from this study demonstrated that, PML colocalizes with Brca1, ATM, Rad51, BLM, and Mre11 at the endogenous levels after exposure to IR. Limited colocalization between PML and Brca1 was found in normal cells, but IR induced a significant relocalization of Brca1 to the PML NBs. To further support a role of PML in DSB-DNA repair, we examined the ability of PML-deficient cells in DSB-DNA repair after cells exposure to IR. Results from this study demonstrated that the efficiency of DSB-DNA repair is significantly lower in the PML-deficient cells. Together, our study strongly supports a role of PML in DSB-DNA repair. The mechanism of how PML promotes DNA repair is unclear, based on the finding by us an others in the field, we hypothesize that PML may play a role in recruiting DNA repair enzymes and organization of the DNA repair complex in respond to IR.

559 Signaling in breast cancer: stopping DNA replication and facilitating DNA repair.

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DNA replication occurs through the coordinated action of multiple proteins, which together form a discrete multiprotein complex in breast cells termed the DNA synthesome. Studies performed with the DNA synthesome isolated from malignant and non-malignant breast cells have demonstrated that the fidelity of DNA replication was significantly reduced in malignant cells when compared to non-malignant cells. Furthermore, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) revealed that proliferating cell nuclear antigen (PCNA), a processivity factor for DNA polymerase δ and essential component of the DNA synthesome, was altered in malignant cells. DNA sequence analysis revealed that the coding sequence for PCNA in malignant cells was identical to the sequence found in non-malignant cells suggesting that the alteration(s) of PCNA occur post-translationally. This structural modification to PCNA present in malignant cells was intriguing not only because PCNA was essential for DNA replication but because PCNA was also required for DNA repair. When cells encounter DNA damage, DNA replication is shut down through the p53-directed increase of $p21^{WAF1}$. Inhibition of DNA replication is mediated through the carboxyl-terminus of $p21^{WAF1}$, which is able to associate with the inter-domain connector loop of PCNA and obstruct the interaction of PCNA and polymerase δ. We have recently found that, the interaction of PCNA and $p21^{WAF1}$ was dependent upon the post-translational state of PCNA. Data also suggest that the ability of $p21^{WAF1}$ to differentially interact with the different isoforms of PCNA may represent a signal transduction pathway that coordinates DNA replication and repair. These findings may therefore clarify the molecular mechanisms by which cells are able to inhibit DNA replication and repair DNA lesions following a DNA damaging event. With the insight gained from the elucidation of these mechanisms we will not only further our understanding of breast cancer, but also using this knowledge, we could ultimately develop better and more practical approaches towards diagnosis and treatment of the disease.

560 Acquired resistance to tamoxifen: functional selection in search for resistance initiating genes (RIGs).

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Background: Selective estrogen receptor modulators (SERMs) are a new class of drugs, which have an important impact in breast cancer treatment and prevention. Tamoxifen (TAM), the first clinically successful SERM, is widely used for treatment of breast cancer. However, TAM-responsive tumors eventually develop resistance to the drug, with poor prognosis for the patient. To identify resistance-initiating genes (RIGs), we developed a novel selection scheme to detect protective effects of introduced genes during TAM treatment.

Materials and Methods: Using a full-length cDNA expression library of 2 million clones, we created a population of MCF-7 cells that expressed all genes of the library. To better imitate in vivo conditions we treated this population with cytotoxic concentrations of tamoxifen (2x10-5M) in estrogen-containing growth media. Recovered cellular clones were tested to confirm their resistance to TAM in clonogenic assay; these clones were also tested for their growth in the presence of other SERMs and the pure antiestrogen ICI 182,780. Recovery of library-introduced genes was done via PCR with vector-specific primers. Insert-specific protective effect is confirmed by re-introduction of recovered genes into naïve population of MCF-7 cells and verification of its increased resistance to TAM.

Results: From library-containing population we recovered several cellular clones that had survived treatment with cytotoxic concentrations of TAM, and exhibited 2-4 fold increase in resistance to this drug. Library-specific genes from these clones (RIGs) were recovered by PCR and sequenced. The ongoing study will demonstrate whether all of the recovered inserts will increase resistance of naïve MCF-7 cells to TAM and other SERMs.

Discussion: Conventional approach to understand the mechanisms of drug-resistance relies on differences in gene expression in parental sensitive cells and their progeny made resistant by gradual increase in drug concentration. In contrast, our approach is designed to identify complete pathways of resistance, including upstream regulators of effector genes that mediate resistance. When TAM-specific RIGs are identified, their expression can be followed during endocrine therapy to evaluate its efficacy and to design chemo-hormonal therapy regimens that prevent development of resistance.

561 Antisense transfection of glucosylceramide synthase modulates gene profiles in response to doxorubicin treatment in drug resistant human breast cancer cells.

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BACKGROUND: Doxorubicin (Adriamycin) is first-line treatment for breast cancer; however, multidrug resistance occurring after treatment greatly limits overall effectiveness. In doxorubicin-selected drug resistant human breast cancer cells (MCF-7-AdrR), several drug resistance-associated molecules, including *mdr1*, mutant p53, and GCS (glucosylceramide synthase) are highly expressed. GCS converts ceramide to glucosylceramide, thus blunting ceramide-induced apoptosis. Antisense GCS (asGCS) blocks GCS gene expression and decreases GCS enzyme activity, and we have shown that asGCS transfection overcomes multidrug resistance in human breast cancer MCF-7-AdrR cells [Liu, Y. Y., Han, T. Y., Giuliano, A. E., and Cabot, M. C. FASEB J. 15, 719-730, (2001)].

MATERIALS AND METHODS: Using cDNA array (Human Apoptosis Q Series), we analyzed apoptosis-associated gene profiles in response to doxorubicin treatment in MCF-7-AdrR and in asGCS transfected MCF-7-AdrR/asGCS cells. GCS and p53 expression levels were also evaluated using RT-PCR and Western blot. Ceramide was measured by [^{3}H]palmitic acid radiolabeling of cells and thin-layer chromatography.

RESULTS: Antisense GCS transfection alone significantly increased the expression of apoptosis-associated genes, including those encoding NFκB, mdm2, Fas (Apo-1), p53, p-21, Caspase-7, and Trail receptor (DR5) in MCF-7-AdrR/asGCS cells, compared to the parent MCF-7-AdrR cells. Doxorubicin treatment (2.5 μM, 48hr) increased ceramide levels in MCF-7-AdrR/asGCS cells by 227% (857 *vs.* 376 cpm, $p<0.001$), compared with MCF-7-AdrR. Consistent with the chemotherapy-sensitizing effect of asGCS transfection, enhanced expression of the apoptosis-associated genes TRAF4, TNFRSF10D, Caspase-6 and Caspase-3 occurred in MCF-7-AdrR/asGCS cells in response to doxorubicin treatment.

DISCUSSION: Antisense GCS transfection modulates gene profiles in MCF-7-AdrR/asGCS cells. Further, doxorubicin treatment enhances cellular ceramide levels and results in enhanced expression of several important apoptosis-associated genes. This study suggests that in addition to inducing apoptosis, ceramide produced via chemotherapy can augment gene expression.

562 Gene expression profiles of intrinsic and induced taxane resistance.

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Background: Our objective was to define genes associated with taxane response to optimize the selection of breast cancer patients treated with this class of chemotherapeutics. Gene expression in tumor samples was analyzed using gene microarrays and correlated with in vitro taxane response determined with the Extreme Drug Resistance™ (EDR) assay.

Materials and Methods: To identify patterns of intrinsic gene expression associated with taxane response, microarrays were performed on cDNA derived from primary tumor specimens classified as either resistant or sensitive in the EDR assay. In parallel with EDR testing, the malignant cells from each tumor specimen were purified (>98%) by fluorescent activated cell sorting (FACS) using monoclonal antibodies directed against oncogene cell surface markers. We also identified sets of genes induced by taxane exposure using three types of specimens: FACS sorted breast tumors, the MCF7 breast cancer cell line, and FACS purified tumor derived vascular endothelial cells. These specimens were exposed to docitaxel (0.1 uM) for 24 hours and positively or negatively sorted into sensitive and resistant subsets using Annexin V as a marker of apoptosis.

Results: Using a t-test analysis for genes differing in expression by >1.5-fold, we found distinct intrinsic and induced gene sets that were significantly associated with taxane response. The intrinsic gene set contained 8 for sensitivity and 25 for resistance. The dynamic induced set contained 12 for sensitivity and 2 for resistance. Few genes were shared between the intrinsic and induced sets.

Discussion: These data support the notion that both intrinsic and induced genes contribute to drug response. Our analysis is designed to find these two groups of genes and determine the different mechanisms at work. We hope to ultimately define gene sets that will make it possible to personalize chemotherapy for individual patients by predicting a given agent's therapeutic effect based on genotyping each patient's tumor.

563 Differential molecular reaction to chemotherapy in resistant and native breast cancer xenograft tumor.

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Background:

Chemotherapy (CHT) resistance is an eminent problem in clinical treatment of breast cancer patients. It often results in treatment failure and recurrent/progressive disease. Few resistance mechanisms have been discovered so far.The aim of our study is to better understand the molecular mechanisms involved and to find gene expression patterns predicting chemotherapy resistance.

Material and Methods:

16 nunu-mice were inoculated (ip) with native (mcf-7) or resistant (NCI/ADR, derived from mcf-7, resistant to anthracyclins) breast cancer cell lines with weekly estrogen. After 5 weeks of tumor growth, 4 animals of each group were treated with CHT (novantrone i.p.), control group received sham injections of NaCL. Animals were dissected two days after CHT and RNA was isolated from tumor samples. To assess differences between the two groups in the moleclular changes induced by chemotherapy, gene expression patterns were analysed before and after CHT using cDNA arrays.

Results:

After CHT, gene expression patterns differ greatly between tumor grown from the native and the resistant cell lines. In native mcf-7 tumor, many genes are downregulated after CHT, while in resistant tumor, massive activation of genes takes place. Resistant phenotype involves expression changes (upregulation) in many genes, suggesting the activation of different pathways to prevent apoptosis.

Discussion:

Differentially expressed genes from the animal experiments will be part of a custom made „breast cancer cDNA array" which is developed at our department for the molecular differential diagnosis of breast cancer.

564 Expression analyses on a model system for invasive breast cancer cells.

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Background: Studies showed that the expression of c-erbB2 correlates with a poor clinical outcome but only a small subgroup of high expression tumours respond to specific therapies. In this high motility subgroup of human breast cancer cells the receptor tyrosine kinase c-erbB2 especially in its heterodimeric form with EGFR is present.

Materials and Methods: As a model we transfected low invasive MDA-MB 468 cells expressing high levels of EGFR but nearly no c-erbB2 with pcDNA3.1+ (Life Technologies, Inc.) based expression-vectors encoding different forms of c-erbB2 using Transfast (Promega). The vectors contained either the cDNA for full length c-erbB2 (MDA erbB2), full length c-erbB2 with a Y to F pointmutation at Y1248 (MDA PM), c-erbB2 lacking the complete intracellular domain (MDA Del) or no insert as a control. Affymetrix U95A High-density microarrays were used to analyse the expression profiles of the low invasion potential (LIP) MDA cells, the PM, Del and neo derivates and the high invasion potential (HIP) cells MDA erbB2 and SK-BR-3 (EGFR and c-erbB2 positive).

Results: Comparing the LIPs to the HIPs we found 30 significantly differentially expressed genes. Protein kinase Cμ (PKCμ), metallothionein 3, RAS interaction / interference protein 1 (RIN1) were found upregulated in the HIPs. Continuing studies are to verify the Chip data by real time RT PCR.

Discussion: PKCμ, RIN1 and metallothionein 3 are potential candidates to be members of the network that regulates motogenic pathway of the invasive cells. Previous uniparametric studies already showed that PKCμ is present in an invasion related complex with other Proteins in the invadopodia of mammary tumour cells. Also metallothionein 3 overexpression was found to be associated with breast cancers having a poor prognosis.

565 Serum PINP, an index of bone turnover, but not bone mineral density, may be predictive of bone metastases in women with primary operable breast cancer.

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Background: It has been postulated that increased bone turnover may be associated with a higher likelihood of skeletal metastases. A trial of adjuvant clodronate vs. placebo in women with primary breast cancer has shown a decrease in the incidence of skeletal metastases (during medication) and a survival gain in the clodronate-treated group. We examined serial BMD and markers of bone turnover measured at entry and during follow-up and their relationship to incident bone metastases.

Materials and Methods: BMD was measured by dual X-ray absorptiometry in 498 women with breast cancer at trial entry (n=243 and 255 for clodronate and placebo, respectively) and repeated annually. Serum biochemical markers of bone turnover (bone specific alkaline phosphatase bAP, carboxy-terminal telopeptide of type I collagen ICTP and n-terminal procollagen peptide of type I collagen PINP) were analysed in stored frozen samples in 559 women at entry and at 1 and 2 years.

Results: Over the two years of treatment, oral clodronate was associated with a significant increase in the mean BMD at the spine and total hip. In contrast, patients in the placebo group demonstrated a significant decrease (differences in mean annual rates of change of +0.016, 95%CI 0.001-0.021g/cm2, p<0.0001 for spine and +0.006, 95%CI 0.002-0.010g/cm2, p=0.004 for total hip BMD). Baseline BMD and changes in BMD at 1 and 2 years were not associated with the subsequent risk of developing skeletal metastases. Treatment with clodronate was also associated with a highly significant decrease in serum PINP at 1 and 2 years. Like BMD, baseline biochemical markers of turnover were not predictive of incident bone metastases. In contrast, serum PINP was significantly higher at 1 and 2 years in those women who developed incident bone metastases during a median of 5.5 years of follow-up. The association at two years remained significant when the analysis was confined to those women with bone metastases diagnosed more than 2 years from entry.

Discussion: Serum PINP may be a useful early marker of bone metastatic disease and may be a tool for selecting patients for long-term adjuvant use of bisphosphonates in women with primary breast cancer.

566 Fibronectin and collagen activate estrogen receptor α and modulate motility in breast cancer cell lines through the c-src pathway.

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Estrogen Receptor positive (ER+) breast cancer cells have a less invasive and aggressive phenotype than ER negative (ER-) cells. In order to investigate the role of ERa as a probable effector of cell adhesion signals, we evaluate ERE-mediated transcription and cell migration under Fn and Col stimulation. In MCF-7 and in MDA MB 231 expressing ERa, both ECM proteins were able to enhance ERE mediated transcription in the same extent of that elicited by E2. The pure anti estrogen ICI 182,780 reversed the up-regulatory effect induced by E2, Fn and Col. Then we asked which functional domain of ERa was affected by the adhesion protein signal and to minimize factors specific to breast cancer cells, we transfected HeLa cells with HEG0 or with two codifying plasmid expressing hER cDNA deleted in the AF-1 or AF-2 domain. We noted that both adhesion proteins are able to enhance transcriptional activity in the presence of AF-1 domain, which appears unaffected by TAM but completely reversed by ICI 182,780.

The activatory effect induced by both ECM proteins is abrogated by PP2 and PD98059 a c-Src and MEK inhibitor respectively.

Cell motility was markedly reduced by both ECM proteins and in a higher extent by E2 in MCF-7 cells and in MDA MB231 expressing ERa. The inhibitory effects caused by either the adhesion proteins or E2 is completely reversed by ICI 182,780.

Similar results were obtained when ER positive breast cancer cells were exposed to both ECM proteins in the presence of an inhibitor of c-Src or a dominant negative c-src. c-Src is know to cause MAPK activation through the well know pathway Raf/Ras/MEK/ERK1/2. So we asked wether C-Src may down regulate estrogen receptor positive breast cancer cell motility by activating Raf/MEK/ERK cascade. We noticed that both ECM proteins were unable to reduce cell motIlity in estrogen receptor positive cancer cells treated with a inhibitor of MK (PD 98059). This datum reinforces the importance of this transductional pathway for a coordinate role of ECM proteins and ER in influencing breast cancer cells motility.

567 Voltage-gated sodium channel expression in human breast cancer cells: possible functional role in metastasis.

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Background: Voltage-gated ion channels are well known to be involved in a variety of cellular behaviours that would be expected to contribute to the metastatic cascade. The present study aimed to evaluate functional aspects of voltage-gated ion channel expression in human breast cancer.

Materials and Methods: A multi-faceted (electrophysiological, pharmacological and molecular biological) approach was used to characterise voltage-activated ion channel expression in two contrasting human breast cancer cell lines: MDA-MB-231 (strongly metastatic) and MCF-7 (weakly metastatic). RT-PCR detection of ion channels in needle biopsies of human breast cancer was also employed.

Results: Functional voltage-gated sodium channels (VGSCs) occurred in MDA-MB-231, but not in the MCF-7 cells, and were blocked by μM tetrodotoxin (TTX). The predominant VGSC expressed was the neonatal splice variant of Nav1.5. In Boyden chamber assays, application of TTX suppressed invasiveness by ~50%. Under control conditions, the endocytic activity of the MDA-MB-231 cells was significantly higher; in the presence of TTX, this difference disappeared. Finally, in a test of 28 biopsies, a high degree of correlation was found between VGSC expression in primary tumour and lymph node metastasis.

Discussion: We conclude that VGSC expression/activity could accelerate the metastatic process in human breast cancer that the neonatal Nav1.5 could be a novel prognostic marker. At present, the mechanism(s) responsible for the VGSC upregulation and the down-stream signalling enabling VGSC activity to be functionally connected to secretory membrane activity and invasiveness are not known.

568 Impact of chromosome 7p gains on gene expression in human breast cancer.

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Background: Cytogenetic, immunohistochemical and cell-biological studies point towards an important role for 7p-gains in a stepwise dedifferentiation of human breast cancer. The responsible, differentially regulated pathways are unknown.

Materials and Methods: Expression profiles of three pairs of tumors, with and without a chromosomal 7p-gain, respectively, were compared by suppression subtractive hybridisation (SSH). All tumors were identical concerning their immunohistochemical ER, PR, Ck 5/6, erbB2, p53 and EGFR-status. Differentially expressed genes selectively amplified by SSH-PCR were cloned, sequenced and identified by NCBI Blast search. Expression of 24 of the putatively differentially expressed genes was quantified in all six specimens by real-time-RT-PCR.

Results: We identified 150 differentially expressed genes by SSH-analysis. Wnt- and TGF-beta-superfamily-pathways were represented by several members. Nearly no proteins participating in apoptotic pathways were found to be differentially regulated. Real-time-RT-PCR analyses revealed the Wnt-receptor FZD6 to have an elevated overall expression in tumors with 7p-gain. Furthermore, a dramatically increased expression of BMPR1B was measured. Together with a high regulation of SMAD7 this points towards an affection of TGF-beta-superfamily signaling. Two of the 7p-gain-tumors showed a remarkably reduced expression of BTF2p44, a subunit of cell-cycle controlling TFIIH.

Discussion: In general, the low expression regulation of apoptose-related factors suggests deregulation of cell-cycle control and proliferation as a major mechanism for growth and accelerated cytogenetic evolution in 7p-gain-tumors. This assumption is further supported by low expression of BTF2p44. Differential FZD6 expression might also lead to an effect on cell-cycle control but this is presumably contradicted by the homogenous beta-Catenin expression measured in all examined tumors. Impact of FZD6 on the cytoskeleton mediated by Rho would instead correspond to the global expression pattern. Altered BMPR1B expression might play a key role in observed extracellular matrix rearrangement and dedifferentiation and thus in 7p-gain-action.

569 Immunohistochemical and genetic alterations in mammary epithelial cells immediately overlying focally disrupted myoepithelial cell layers.

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Background: Our previous studies, using a double immunostaining technique with antibodies to smooth muscle actin and estrogen receptor (ER), revealed that focal losses of ER expression in epithelial (EP) cells and disruptions of the subjacent myoepithelial (ME) cell layers were correlated events in ER (+) non-invasive breast lesions. This study attempted to confirm this finding and to assess the genetic profiles of cells overlying disrupted ME cell layers.

Materials and Methods: Tissue sections were made from 220 patients with various types of breast lesions and were double immunostained with the same protocol. Cross sections of ducts lined by ≥ 40 EP cells were examined for focal ME cell layer disruptions, defined as an absence of ME cells, resulting in a gap equal to or greater than the combined size of 3 EP or ME cells. EP cells immediately overlying disrupted ME cell layers and adjacent EP cells within the same duct were microdissected for loss of heterozygosity (LOH) and microsatellite instability (MI) assessment.

Results: Of 5,698 duct cross sections examined, 405 focal disruptions were detected. Of which, 350 (86.4%) were subjacent to cells with a loss of ER expression, while 55 (13.6%) were subjacent to cells with a high level of ER expression. A vast majority of ER (-) cells immediately overlying disrupted ME cell layers displayed a substantially higher frequency or different pattern of LOH and MI, compared to adjacent ER (+) cells within the same duct, while in a small proportion of cases, ER (-) cells showed a marked lower or even no distinct genetic alterations.

Discussion: These results suggest that a vast majority of ER (-) cells overlying disrupted ME cell layers represent an altered clone that may be in the process of early invasion, while a few of these may be involved in a normal expansion or replenishment of the duct.

Supported by the US Army Medical Research and Materiel Command under DAMD 17-01-1-0129 and DAMD 17-01-1-0130 to Yan-gao Man, MD., Ph.D.

570 HER2-overexpressing human breast cancer xenografts exhibit increased angiogenic potential mediated by vascular endothelial growth factor (VEGF).

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VEGF is an important endothelial mitogen involved in development and differentiation of the vascular system. As a secreted heparin-binding glycoprotein, VEGF has multiple effects on endothelial cells including increased vascular permeability, enhancing cell proliferation and migration, and inducing release of proteinases involved in tumor invasion. These observations suggest VEGF as an important molecule regulating angiogenesis in human cancer. A role of VEGF in breast cancer progression is evident from clinical studies demonstrating elevated serum VEGF in patients with invasive breast cancer. VEGF in breast tumor cytosols is correlated with microvessel density, as well as with disease-free and overall survival in primary breast cancers. HER2 proto-oncogene is amplified/overexpressed in 20-25% of human breast carcinomas and is associated with poor clinical prognosis. We provide evidence that increased expression of VEGF may in part mediate the aggressive phenotype of human breast cancer cells that overexpress HER2 by increasing their angiogenic potential. In transfection studies, engineered overexpression of HER2 is associated with increased expression of VEGF in human breast cancer cells at both the RNA and protein levels in vitro. Moreover, exposure of these cells to recombinant heregulin significantly increases VEGF protein secretion into conditioned media; whereas exposure to anti-HER2 antibody trastuzumab significantly decreases VEGF specifically in HER2-overexpressing cells. Utilizing intravital microscopy, HER2-overexpressing breast cancer cells exhibit increased angiogenic potential in vivo compared to control cells. Taken together these data indicate that VEGF is a downstream target of the HER2 signaling pathway. In HER2-overexpressing human breast cancer xenografts, combined treatment with trastuzumab plus humanized anti-VEGF antibody (bevacizumab) in vivo results in significant reduction in xenograft volume compared to single agent control. These data implicate VEGF in the aggressive phenotype exhibited by HER2-overexpressing tumors and support the use of combination therapies directed against both HER2 and VEGF for treatment of breast carcinomas that overexpress HER2.

571 Upregulated and active HER2/neu and ER α converge in tamoxifen-stimulated endometrial cancer.

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Backgound:Tamoxifen (Tam) is the endocrine treatment currently being used to treat all stages of estrogen receptor (ER) positive breast cancer and as a chemopreventive for high risk women. Five years of Tam treatment increases the incidence of endometrial cancer. This study investigates the relationship between overexpression of HER2/neu and ERα in an in vivo model of Tam-stimulated endometrial cancer (EnCa101TAM) in athymic mice.

Material and Methods:Tam-stimulated endometrial tumors were developed in athymic mice by serial passage of Tam-naive tumors (ECC-1) with estrogen (E2), then with 1.5 mg Tam orally by gavage for more than 5 years. Expression of human EGFR, HER2/neu, HER3, and HER4 mRNA was measured using Real-Time RT-PCR. Western blot and immuno-precipitation (IP) were done to detect protein and the extent of tyrosine phosphorylation of both EGFR and HER2/neu. CoIP was performed to evaluate the interaction between ER α and HER2/neu.

Results:The results demonstrate that EnCa101TAM that are stimulated to grow with Tam (>5 years) express 11 fold higher HER2/neu mRNA compared to ECC-1 tumors that are growth stimulated by estrogen but inhibited by Tam. Either short (2 months) or long term (>3 years) treatment of EnCa101TAM tumors with E2 did not significantly affect HER2/neu mRNA levels. Western blot and IP show that levels of HER2/neu protein and the extent of tyrosine phosphorylation at Y1248 are significantly increased in EnCa101TAM tumors-treated with either Tam or E2 for more than 3 years compared to the parental ECC-1 tumors. In contrast, levels of phosphorylated EGFR and total EGFR protein are increased in the ECC-1 compared to EnCa101TAM tumors. Interestingly, levels of ER α protein are dramatically upregulated in the EnCa101TAM tumors-treated with either Tam or E2. CoIP reveals that ERα interacts with phosphorylated HER2/neu in the EnCa101TAM tumors-treated with Tam or E2.

Discussion: Results suggest that transcription of HER2/neu is disregulated in EnCa101TAM tumors, upregulated HER2/neu protein in EnCa101 tumors is active as evidenced by the measure of phosphorylated Y1248, and the overexpressed ERα protein preferentially interacts with the active form of HER2/neu protein indicating a direct role for active HER2/neu in the ERα pathway in Tam-stimulated endometrial cancer.

572 HGF disrupts tight junction function of human breast cancer cells which can be reversed by NK4, the HGF-antagonist.

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Background: Tight junctions in epithelial cells act as cell-cell adhesion structures and govern paracellular permeability. Disruption of these functions often leads to the dissociation of cancer cells. NK4 is the HGF-antagonist that we have shown to prevent HGF-induced changes in tight junctions of endothelial cells. This study aimed to determine whether (i) HGF is able to modulate the expression and function of tight junction molecules in human breast cancer cell lines (MB MDA 231 and MCF7) and (ii) if these changes could be inhibited by NK4.

Methods: Functional changes were monitored using transepithelial resistance (TER) and paracellular permeability (PCP), mRNA and protein level changes monitored using Q-PCR and western blotting.

Results: HGF decreased TER over 2 h (from 100% to reduction of 50% in MDA and 68% in MCF7). Addition of NK4 prevented HGF induced decrease in TER (increase of 121% in MDA and 116% in MCF7, p=0.04, n=3). PCP of both breast cancer cell lines was increased on co-culture with HGF (253+/-6 RFU and 353+/-4 for MDA and MCF7 respectively in the control, to 3220+/-2; 662+/-5 with HGF. Again, addition of NK4 inhibited the action of HGF. Q-PCR showed that HGF modulated levels of tight junction molecule mRNA: ZO-1 and Occludin was increased in MDA after 1h incubation with HGF, returning to initial levels by 24h (ZO-1 levels; 293 copies/50 ng RNA at 0h, 1790 at 1h, 691 at 24h: Occludin levels; 1330 at 0h, 5670 at 1h, 1450 at 24h). Expression of both Claudin-1 and 5 were decreased over 24h incubation; Claudin-1: 15.9 copie/50 ng RNA at 0h, 4.61 at 24h; Claudin-5: 117 at 0h, 35.4 at 24h. Similar trends were observed in MCF7 cancer cells. Western blotting confirmed these changes.

Conclusion: We report that HGF is able to disrupt the function of tight junctions in human breast cancer cells, due to changing levels of tight junction molecules. HGF-induced changes in tight junction function can be prevented by the HGF-antagonist NK4 and may therefore have a role to play in inhibiting cancer cell dissociation.

573 EphA2 overexpression in breast cancer decreases estrogen dependence.

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EphA2 is a receptor tyrosine kinase found at low levels in normal adult breast epithelia but frequently overexpressed in malignant breast tissue. EphA2 overexpression transforms MCF-10A cells in both in vitro and in vivo models. In breast cancer cell lines EphA2 overexpression correlates with aggressiveness and metastatic potential and is inversely correlated with estrogen receptor (ER) expression. In the present study, we investigate the impact of EphA2 overexpression on the growth and estrogen dependence of ER+ MCF-7 cells. In the presence of supplemental estrogen, MCF-7 cells stably transfected with excess EphA2 (MCF-7/EphA2) grow more rapidly in vitro (2-fold difference measured by soft agar growth assay, $p<0.01$) and in vivo (mean tumor size 747 mm3 vs. 1119 mm3; $p=0.031$). ER activity is decreased in MCF-7/EphA2 cells compared to parental MCF-7 cells. MCF-7/EphA2 cells proliferate in vitro and form tumors in athymic mice in the absence of supplemental estrogen (maximum tumor size 4.5 mm3 vs. 40.7 mm3; $p=0.0039$). Similarly, MCF-7/EphA2 cells are less sensitive to tamoxifen in vitro (colony survival in soft agar 40% MCF-7 vs. 90% MCF-7/EphA2); evaluation of tamoxifen sensitivity in vivo is ongoing. In 2D and 3D tissue culture systems, treatment with an EphA2-directed antibody that mimics ligand binding reverses the malignant phenotype and restores estrogen dependence and tamoxifen sensitivity. These studies suggest that EphA2 overexpression renders breast cancer cells less hormone dependent; EphA2 directed antibodies restore estrogen sensitivity in vitro. As such, EphA2 represents a potential target for future breast cancer therapies.

574 Interleukin-7 (IL-7) and IL-7 receptor expression in breast cancer.

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Introduction:
Interleukin-7 (IL-7) stimulates the proliferation and differentiation of progenitor B and T cells. Although it is known to induce differentiation and proliferation of some hematological malignancies including certain types of leukemias and lymphomas, little is known about its role in solid tumors including breast cancer. We studied the effects of IL-7 and its receptor (IL-7R) on breast cancer and endothelial cells, and its expression in a group of patients with breast cancer.
Methodology:
Breast cancer cell lines (MDA MB 231 & MCF-7), endothelial cell line, pheripheral mononuclear and polymorphonuclear cells, stromal fibroblasts, a leukaemia cell line (HL60) and breast cancer cDNA were analysed for the expression of both IL-7 and IL-7R using RT-PCR. β-actin used as a housekeeping gene. Breast cancer tissues (n=108), background tissues (n=38) and normal breast tissues (n=8) were analysed for IL-7R and IL-7 expression using real time quantitative PCR.
Results:
IL-7R was expressed in breast cancer cells, MDA MB 231 and MCF-7, endothelial cell, peripheral mononuclear and polymorphonuclear cells. IL-7 itself was expressed only in mononuclear cells, polymorphonuclear cells, and a leukaemia cell line (HL60). The number of IL-7R transcript copies was significantly higher in tumor tissues and its background tissues than in normal breast tissues. The highest level of IL-7R was in ductal carcinoma followed by lobular, mixed, and mucinous types. Histopathological grade 3 had a higher level of IL-7R (26±6.4 copies/50ng RNA) compared with grade 2 (21.9±9.4) and grade 1 (14.3±6.3). TNM3 had a higher level of IL-7R (40.2±6.3) followed by TNM2 (25.92±8.4) and TNM1 (18.1±6.2). Furthermore, the highest level of IL-7R was in patients with NPI3 (Nottingham Prognostic Index 3) (48.0±24.9 copies of IL-7R transcript/50ng RNA), than those with NPI2 and NPI1 (21.8±8.4 in NPI2 and 16.0±3.3 in NPI1). IL-7 expression was not detected in tumor and normal breast tissues.
Conclusion:
Expression of IL-7 receptor was found to be elevated in breast cancer tissues and the levels of expression are associated with tumor size and nodal involvement of breast cancer.

575 Cyclin I is closely associated with VEGF and KDR expression in human breast cancers.

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In a large subtractive screen of a transformed breast cancer cell line and its normal counterpart we have identified several differentially expressed cDNAs. One of these, the T5C11 was later found to be identical to a new member of the cyclin gene family now known as cyclin I. This gene includes a cyclin box which encodes a protein fold responsible for binding to cyclin dependent kinases (Cdks). Our immunoprecipitation results indicate that cyclin I gene product can potentially bind Cdk2. The cyclin I gene was localized to chromosome 4q21. The cyclin I mRNA is expressed in skeletal muscle, heart, brain and other differentiated tissues but not in the normal human breast epithelium. The dysregulation of cyclins and other cell cycle regulators have been implicated in oncogenesis and knowledge of aberrations in cell cycle regulatory gene products is most likely of central importance in understanding the transformation process.
In a material of 87 invasive breast cancers arranged in a tissue-array system, with known status of cell cycle aberrations and clinico-pathological data, the expression of cyclin I was monitored using immunohistochemistry. The antibody used was a poly-clonal cyclin I specific antibody recognizing the overexpressed form of cyclin I by Western blotting validating the analyses. Cytoplasmic staining of variable intensity was observed in most of the tumors 30.2% had low staining, 43.0% with intermediate staining and 19.8% had strong staining whereas 7% lacked cyclin I protein. Variable nuclear staining was also observed and 22.1% of the tumors showed intermediate or high nuclear cyclin I expression. There was no obvious correlation between nuclear or cytoplasmic cyclin I staining and the presence of aberrations in cyclin D1, cyclin E, p27, p16, p53, pRb, c-myc, c-erbB or bcl-2 or standard clinico-pathological parameters including survival. However, a strong association was observed between cytoplasmic cyclin I staining and VEGF ($p=0.001$) as well as to the VEGF receptor KDR ($p=0.001$), suggesting a link between cell cycle regulators and angiogenesis. In summary, our results indicate that cyclin I is expressed in breast cancer samples and might contribute to the transformation process via interaction with Cdk2 or through cellular pathways associated with angiogenesis.

576 Cytogenetic alterations and cytokeratin expression patterns in breast cancer - integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis.

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Background: The introduction of a concept proposing multiple cellular subgroups in the normal female breast, including cytokeratin (Ck) 5/6 positive progenitor-cells (Boecker et al. Lab Invest 2002) offers a new explanation for the existence of highly-aggressive breast cancers with and without Ck 5/6-expression.
Materials and Methods: Using the tissue microarray technique, 166 breast cancer cases, all characterised by Comparative Genomic Hybridization (CGH) were evaluated by immunohistochemistry, using 15 different antibodies (estrogene receptor, progesterone receptor, p53, Ki-67, erbB2, Epidermal Growth Factor Receptor, Cyclins A, D1, E, bcl-2, p21, p27, Ck 5/6, Ck 8/18 and smooth muscle actin), and Chromogenic-in-situ-Hybridization (CISH) for erbB2. Biomathematical cluster analysis was applied to confirm the conventional interpretation of the results by an independent approach.
Results: Ck 5/6 positive breast carcinomas in general were ER and PR negative, highly-proliferating (as reflected by Ki67 and cyclin A), and were associated with specific protein expression patterns, such as expression of p53, and EGFR, which could further be demonstrated by biomathematical cluster analysis. In contrast Ck 5/6 negative breast carcinomas revealed a lower tumour proliferation rate, an increased expression of p21, p27, erbB2 and bcl-2 and a significantly lower number of genetic alterations with losses of chromosomal material of 16q as the most common genetic alteration. A significant difference between erbB2- or p53-overexpressing carcinomas and carcinomas expressing Ck 5/6 could not be demonstrated.
Conclusions; Our data give first hints to the hypothesis that different cellular subgroups in the female breast give rise to subgroups of breast carcinomas with differing protein expression and cytogenetic alteration patterns.

577 Subgroups of breast cancer determined by estrogen receptor and cyclin D1 expression demonstrate different behavior and genetic abnormalities.

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The cell cycle machinery is regulated by cyclin dependent kinases and a set 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. Cyclin D1 is one of the main positive regulators of the G1/S transition and the gene product is overexpressed in approximately 25 % of all breast cancer. Besides that cyclin D1 can effect cdk4/6 it also have cdk-independent functions and can directly activate the estrogen receptor as a co-factor. To understand the interplay between cyclin D1 and the estrogen receptor, we have detailed the protein contents in large materials of primary breast cancer samples as well as characterized their relation in tumors with heterogeneous ER expression. Interestingly, we observed a clear inverse association between cyclin D1 and the ER in a fraction of the tumors. Tumors with extreme cyclin D1 levels had in general lower ER content whereas tumors with high ER had low cyclin D1. Tumors within different cyclin D1/ER clusters also varied regarding clinico-pathological parameters as differentiation, grade, proliferation, cerb-B2 content, survival and age, suggesting that the clusters represened different types of tumors. Some tumors with heterogeneously expressed ER also showed an inverse association between tumor cells with lower ER content and high cyclin D1 protein due to gene amplification. In summary we propose that the intimate interaction between the ER and cyclin D1 in breast cancer might be of importance for resistance for antihormonal treatment. Other reasons for ER heterogeneity in breast cancer will also be discussed.

578 Focal adhesion kinase (FAK) as a marker of malignant transformation in breast epithelium.

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Introduction: Integrins mediate cell adhesion to extracellular matrix and stimulate signaling events involved in cell proliferation, survival and migration. Focal adhesion kinase (FAK) is considered to be the central molecule in integrin-mediated signaling. Previously, FAK has been implicated in invasive tumor behavior based on Northern and Western blotting using total tumor tissue homogenates.

Material & Methods: In this study we used immunohistochemistry to correlate FAK expression with cell phenotype in *in situ* (n=7) and invasive ductal carcinoma (n=8) of the breast. All invasive carcinoma cases were of high nuclear and histologic grade. As a control group, we used samples of normal breast epithelium (n=11). FAK staining was graded as negative when no or faint staining was observed. All samples with moderate to strong staining for FAK were considered positive.

Results: The FAK expression was negative in all cases of normal breast epithelium. All eight cases of *in situ* breast carcinoma, and six out of eight cases of invasive ductal carcinoma were positive for FAK. There was no statistically significant difference in FAK expression between *in situ* and invasive carcinomas (p=0.6). However, there was a significant difference in FAK expression between the benign and the neoplastic epithelium (p<0.001).

FAK expression as a marker of malignant transformation in breast tissue

FAK Expression	Normal Epithelium	*In Situ* Carcinoma	Invasive Carcinoma	All Carcinoma
Negative	11/11	0/7	2/8	2/15
Positive	0/11	7/7	6/8	13/15

Normal epithelium vs. all carcinoma, p<0.001; *in situ* vs. invasive carcinoma, p=0.06

Discussion: While the earlier studies using whole tissue homogenates concluded that FAK is overexpressed primarily in invasive tumors, we found that FAK is also overexpressed in *in situ* breast carcinomas. Thus, FAK appears to be a marker of malignant transformation, rather that being a marker of invasive tumor behavior.

579 Purification and functional characterization of breast cancer cell poly(ADP-ribose) polymerase.

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The genetic damage which accompanies the development and progression of breast cancer has been linked to defects in the DNA replication and repair processes in these cells. We have previously isolated an intact, stable, and fully functional multiprotein DNA replication complex (designated the DNA synthesome) from a variety of non-malignant and malignant tumor cells and tissues including breast cancer cells. All of the components necessary for DNA replication, including poly(ADP-ribose) polymerase (PARP), have been detected in the DNA synthesome. We have shown that the malignant breast cell DNA synthesome exhibits a 6-8 fold decrease in the replication fidelity relative to the non-malignant breast cell DNA synthesome. In addition, the transformation of a non-malignant human breast epithelial cell to a malignant state is accompanied by a significant alteration in the mobility of specific protein components of the DNA synthesome (such as proliferating cell nuclear antigen, PCNA) following 2D-PAGE of the replication complex. PARP has long been implicated in the processes of DNA replication, DNA repair, and cellular transformation. The unique form of PCNA found exclusively in malignant breast cells lacks the poly(ADP-ribose) modification which is found in the non-malignant form of the protein. In order to establish whether the malignant transformation process is accompanied by an alteration in the syntheome-associated PARP, the DNA synthesome isolated from non-malignant (MCF-10A) and malignant (MCF-7) breast cell lines was resolved by 2D-PAGE and the migration pattern as well as the isoelectric point (pI) were determined. In addition, we isolated PARP from both non-malignant and malignant breast cell lines using phosphocellulose and affinity column chromatography. PARP has been shown to be enriched during its purification. We are currently comparing the physical characteristics of PARP isolated from malignant and non-malignant breast cells in order to explain how poly(ADP-ribosylation) of DNA synthesome components contribute to the observed decrease in replication fidelity.

580 Mechanisms of formation and therapeutic resistance of breast carcinoma lymphovascular emboli.

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Background: Lymphovascular tumor emboli are a marker of breast carcinoma aggressiveness, recurrence, metastasis and chemotherapy / radiotherapy treatment failures.

Materials and Methods: Using an *in vivo* and *in vitro* model of lymphovascular emboli formation, we have gained insights into the mechanisms of both their formation as well as their resistance to therapy.

Results: Lymphovascular emboli form on the basis of an intact and overexpressed E-cadherin / α,β-catenin / actin axis and decreased degree of sialylation of MUC1. This resulting tight ball of tumor cells resists radiation and chemotherapy-induced apoptosis for the following reasons: 1) the center of the emboli are hypoxic as evidenced by pimonidazole uptake studies; 2) the periphery of the emboli do not exhibit a Rb, p21 or p27 mediated growth arrest and hence, while susceptible to the damaging effects of radiation and chemotherapy from the standpoint of the cell cycle, exert protective bystander effects on neighboring tumor cells. Disadherence of the lymphovascular embolus by differing immunological (anti-E-cadherin) , proteolytic (trypsin), cation removal (- Ca ++) and gene transfer (dominant negative E-cadherin mutant; fucosyl transferase) strategies all induce apoptosis measured by TUNEL and flow cytometry by a common yet seemingly novel apoptotic pathway determined by gene chip analysis. This novel apoptotic pathway differs from classical anoikis. These same disadherence strategies also potentiate the apoptosis-inducing effects of both chemotherapy and radiation therapy which previously were ineffective at inducing apoptosis of intact emboli. The mechanism of this apoptosis potentiation suggested by gene chip analysis is different from the mechanism of apoptosis induction.

Discussion: Lymphovascular emboli, in the final analysis, are the major cause of morbidity and mortality from breast cancer. Therapeutic strategies targeting both their formation and neutralizing their resistance to therapy would be highly desired in future rationale drug design approaches.

581 Dehydroepiandrosterone-sulfate inhibits estrogen/ progesterone-negative breast cancer cells via the androgen receptor.

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Background: We previously demonstrated that ER/PR positive breast cancer cells proliferate when exposed to the adrenal androgen dehydroepiandrosterone-sulfate (DHEA-S) despite treatment with anastrozole, tamoxifen, or Faslodex. We now investigate the effect of DHEA-S on an ER/PR negative line alone and in the presence of Casodex, an anti-androgen agent.

Materials and Methods: HCC1937 breast cancer and LNCaP prostate cancer cells treated with 100uM anastrozole were stimulated with 22.8uM DHEA-S. Separate cells were exposed to DHEA-S, 0.1nM tamoxifen, 100uM anastrozole, and 0.1uM-10uM Casodex. Finally, HCC1937 cells treated with 100uM anastrozole and 0.1nM-500uM Casodex were stimulated with DHEA-S. Cell growth was calculated via MTT assays. Presence of AR was confirmed by RT-PCR.

Results: HCC1937 cells treated with anastrozole were inhibited to -22% following DHEA-S exposure, while LNCaP cells showed proliferation of 26%. Presence of tamoxifen, anastrozole, and 10uM, 1uM, or 0.1uM Casodex yielded growth of 0%, -7%, and -17% in the breast line. Identically treated LNCaP cells demonstrated growth of -22%, 6%, and 7% respectively. HCC1937 cells treated with DHEA-S, anastrozole, and Casodex showed growth recovery at all Casodex concentrations, ranging from -4% to -13%.

Conclusions: Adrenal DHEA-S exposure inhibits growth of AR positive, ER/PR negative HCC1937 breast cancer cells. Suppression was reversed when cells were treated with Casodex, implicating the AR in breast cancer growth regulation. These findings suggest that AR manipulation may prove clinically beneficial and warrants in vivo trials.

582 Hypoxia-induced phenotypical changes in ductal breast carcinoma in situ.

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Hypoxic cells in solid tumors adapt to low oxygen pressure by changing their gene expression program to promote neovascularization, which involves the stabilization and activation of hypoxia inducible transcription factors. In the sympathetic nervous system tumor, neuroblastoma, hypoxia induces an immature tumor cell phenotype with neural crest-like characteristics. Using mammary ductal carcinoma in situ (DCIS) as a model system, we tested whether hypoxia-induced dedifferentiation is a phenomenon restricted to neuroblastoma, or if it occurs in other tumor forms as well. In DCIS tumor lesions, there is a hypoxic zone of cells with high HIF-1α protein levels surrounding the central necrosis. In general, the studied tumors were of low differentiation grade, but a fraction of the DCIS lesions contained cells that had retained some ability to polarize. In such lesions, the tumor cells were dedifferentiated with diminishing distance to the necrosis, i.e. they had lost their polarization and the nucleus - cytoplasm ratio was increased. The change toward a less mature morphological phenotype was further manifested by a change in the differentiation marker gene expression pattern. The changes in gene expression also occurred in cultured hypoxic breast cancer cells suggesting that hypoxia induces dedifferentiation in DCIS cells.

583 HIF-1α/CAIX coexpression in invasive human breast cancer.

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Tumour hypoxia is one of the hallmarks of solid tumours, often associated with decreased efficacy of therapy. Furthermore, hypoxia is powerful trigger for angiogenesis and associated with tumour progression. In general cellular hypoxia triggers a broad response that is primarily mediated by the hypoxia inducible factor (HIF-1). From our previous studies we concluded that the level of HIF-1α increases during breast carcinogenesis, especially in poorly differentiated lesions, and is associated with poor prognosis in lymph node negative patients.

We also suggest that HIF-1 overexpression in breast cancer may also be oncogene-related, as we have observed diffusely HIF-1α-overexpressing tumours without (hypoxic) necrosis.

Tumour cells may survive the hypoxic stress by creating an acidic environment that protects mitochondria. Carbonic anhydrases that reversibly convert carbon dioxide and water to carbonic acid, may play a role in this process. Interestingly carbonic anhydrase 9 protein (CAIX) was found to be a tumour marker in various tumours and even more interestingly the expression of CAIX is regulated by HIF-1α, and has been shown to be associated with poor prognosis in breast cancer. In the present study we examined the expression of CAIX and HIF-1α in a group of 30 randomly selected invasive breast cancer cases by brightfield immunohistochemical single staining (of serial sections) and double staining (the CAIX and HIF1α positive cases) using monoclonal antibodies to HIF-1α and CAIX.

Having observed colocalization of HIF-1a and CAIX in the single stained serial sections in many cases, we confirmed HIF-1α/CAIX coexpression in the same cells, predominantly in peri-necrotic areas (33%). Rarely, expression of either HIF-1a or CAIX was seen in non-perinecrotic areas.

The results support the hypothesis that HIF-1a expression is regulated by two different pathways 1) hypoxia mediated, visible as peri-necrotic HIF-1α expression with concerted CAIX expression and 2) oncogene/ tumour suppressor gene regulated, that results in diffusely HIF-1α expressing tumours. The absence of expression of HIF-1α target genes in diffusely HIF-1α expressing tumours suggests that these tumours may be less aggressive than those harboring necrosis. CAIX may be a more useful marker of tumor hypoxia than HIF-1α.

584 Alpha6beta4-integrin-mediated phosphorylation of insulin receptor substrate 1 in fine-needle aspirates of breast cancer.

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Background: As the principal cell surface receptors for extracellular matrix proteins, integrins may play an important role in tumor cell invasion and metastasis. Ligation of α6β4 is known to activate phosphoinositide 3-OH kinase (PI3K) in vitro, and activation of PI3K is associated with increased invasiveness of breast carcinoma cell lines. The activation of PI3K following ligation of α6β4 has recently been shown to be mediated by insulin receptor substrate 1 (IRS-1). We were interested in determining whether α6β4-mediated signal transduction could be measured in fresh tumor cells isolated from surgical specimens by fine-needle aspiration.

Materials and Methods: Tumor cell suspensions were prepared from fine-needle aspirates of 15 resected breast specimens with invasive ductal carcinoma. Tumor cell purity was assessed by morphologic evaluation of cytologic preparations, and cell viability was measured by Trypan blue exclusion. Tumor cells were stimulated in suspension with either anti-α6 antibody or anti-IgG control for 5 minutes at 37°C, and cytospin preparations were made. Immunohistochemical staining for phospho-IRS-1 was then performed on the α6-stimulated cells and control cells, and staining results were correlated with α6β4 expression as measured by immunohistochemical staining for the β4 subunit.

Results: Median tumor cell purity was 93%, and median viability was 94%. Seven of the 15 cases showed either low or high α6β4 expression. Increased phospho-IRS-1 was detected in 3 of these 7 cases following stimulation with anti-α6 antibody, and each of these 3 cases showed high α6β4 expression. No increase in phospho-IRS-1 was detected in the α6β4-negative cases following stimulation with anti-α6.

Discussion: These findings demonstrate that: (1) integrin-mediated signal transduction can be meaured in tumor cells prepared from fine-needle aspirates, and that (2) α6β44-mediated phosphorylation of IRS-1 occurs not only in breast cancer cell lines but also in fresh tumor cells obtained from breast cancer specimens. Future studies should determine whether integrin-mediated signal transduction in breast cancer specimens correlates with clinical outcome.

585 Altered expression of heat shock proteins in breast cancer cells growing 3-dimensionally in a rotary cell culture system.

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The rotary cell culture system (RCCS), developed by NASA, is a fluid-filled vessel with a gas-permeable membrane that maintains cells in culture in constant suspension. This allows cells to associate and grow in three dimensions. We examined the effects of these culture conditions on tumor cell lines, including human breast cancer. Compared to adherent monolayers formed by the cancer cells grown in standard tissue culture vessels, nonadherent multicellular structures were formed in the RCCS that were composed of cells bound closely together with tight junctions. These structures did not appear to be random cell aggregates, and instead, displayed some polarity as evident by the presence of sinus-like cavities observed with scanning electron microscopy. When analyzed by flow cytometry, tumor cells grown in the RCCS displayed levels of MHC Class I, CD54 and CD58 that were comparable to standard cultures. However, MHC Class II (HLA-DR) expression was reduced in cells grown in the RCCS. On the other hand, these cells displayed increased heat shock proteins (Hsp), including Hsp70 and Hsp27, at levels approaching those observed in monolayer cultured cells subjected to sublethal heat shock. Despite similarities in Hsp upregulation, some differences between RCCS-grown and heat-shocked monolayers were observed, i.e. the heat-treated monolayer cells were more sensitive to lysis by innate effector cells and displayed resistance to doxorubicin (compared to untreated monolayer controls), while RCCS-grown tumor cells remained sensitive to doxorubicin and displayed decreased sensitivity to lysis by cytotoxic cells (compared to the same controls). Furthermore, heat shock did not reduce HLA-DR expression on monolayer cultured cells. Increased Hsp, downregulation of HLA-DR, and resistance to host immunity have been observed for tumors growing *in vivo*. Our data therefore suggest that the RCCS mimics the *in vivo* situation, and that this culture system might be an appropriate model for evaluating the role of Hsp in drug-sensitivity, the malignant process, and the immune response to cancer.

Supported by NASA grant NAG8-1347.

601 Nonpalpable breast carcinoma: superior survival when compared with palpable breast cancer.

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Background: It remains controversial as to whether mammographically detected invasive cancers have a better long-term survival when compared with palpable lesions.

Materials and Methods: From 1979 to 2002, we treated 3,617 patients with breast cancer: 1564 patients had nonpalpable, mammographically discovered lesions and 2053 patients had palpable lesions. Survival data between the two groups were compared.

Results: Of 1564 nonpalpable carcinomas, 576 were invasive and 988 (63%) were in situ. 498 (32%) patients with nonpalpable lesions were treated with mastectomy. The remaining 1066 (68%) were treated with various forms of breast conservation. 297 axillary dissections were done in patients with noninvasive lesions. Only 2 patients (0.7%) had positive nodes by H&E. 30 of 201 (15%) node dissections in patients with nonpalpable invasive cancer contained metastases by H&E. 16 patients with nonpalpable breast cancer have developed distant metastases, 11 of whom have died of breast cancer. The impact of mammography is appareciated when these 1564 patients with screen-detected nonpalpable breast cancer are compared with 2053 patients with palpable lesions. The 10-year actuarial distant disease-free, cause-specific, and overall survival is shown below. Comparison reveals that patients with nonpalpable breast cancer have a significantly higher distant disease-free, cause specific, overall survival than patients with palpable breast cancer (all P values <0.001).

Discussion: Image-detected breast biopsy of nonpalpable mammographically suspicious areas yield a subgroup of breast cancer patients with a potential cure rate of 95% at 10 years, a subgroup that is statistically superior to patients who present with palpable breast cancer.

10-Year Kaplan-Meier	All Nonpalpable N = 1564	Invasive Nonpalpable N = 576	All Palpable N = 2053
Distant Disease-Free	95%	89%	73%
Cause-Specific Survival	97%	93%	77%
Overall Survival	89%	84%	70%

602 The importance of annual mammography screeening in women diagnosed with ductal carcinoma in situ.

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Background: Controversy continues over the benefit of annual screening mammography. In addition to the impact on survival, the detection of smaller, more differentiated tumors by annual mammography screening may provide the benefit of less aggressive, cost-effective, local treatment options for women diagnosed with ductal carcinoma in situ (DCIS).

Materials and Methods: Retrospective chart review was performed on women diagnosed with DCIS. 146 women were diagnosed with DCIS by follow up mammogram. For these women, the interval between mammograms was defined as the time in months between the diagnostic mammogram and the previous screening mammogram. Pathologic DCIS size in millimeters was determined by the largest DCIS dimension seen on one slide. Pathologic DCIS grade was defined as low, intermediate or high.

Results: Women diagnosed with DCIS by follow up mammography who were age 50-59 (n= 53) had an average duration between mammograms of 1.3 years, whereas women < 50 (n=47) and > 60 (n=46) had an average duration between follow up mammograms of 2.1 years and 2.0 years, respectively (p=0.001). Women < 50, who had a >12 month mammography interval, had increased pathologic DCIS size (p=0.04); women > 50 displayed a similar trend. Women <50, diagnosed with intermediate or high grade DCIS lesions, had a longer average duration between mammograms (2.5 years) than those with low grade DCIS (1.5 years). For women > 50, those with intermediate or high grade DCIS lesions, had an average duration between mammograms of 1.8 years compared to women > 50, with low grade DCIS, whose average duration was 1.0 year (p=0.006).

Discussion: Women 50-59 diagnosed with DCIS more closely followed annual mammography screening recommendations, while women < 50 and > 60 had statistically significant longer mammography intervals between screening mammograms. Annual screening mammography in women leads to the detection of pathologically smaller, lower grade DCIS lesions, which may require less aggressive local treatment.

603 Clinical and histopathologic features of mammographically occult breast cancer.

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Background: Mammographically occult breast cancer is a diagnostic dilemma that carries medical, psychosocial and legal iterations. A false negative mammogram can be defined as a nondiagnostic mammogram within one year of a new breast cancer diagnosis. Prior studies have estimated a false negative rate of mammography of approximately 15%, and because these breast cancers are usually palpable at the time of diagnosis, they are reported to be at a more advanced stage. The purpose of this study was to compare demographic and histopathologic parameters of patients who had mammographically occult breast cancer as opposed to those who had mammographically detectable lesions at diagnosis. Methods: We performed a retrospective review of the Cleveland Clinic Breast Center database of women newly diagnosed with breast cancer from 1997-2001. Variables that were collected in the analysis included: age, race, diagnosis, tumor size, lymph node status, HER-2/neu status, ER/PR status and menopausal status. Differences between groups were evaluated using the Chi-square test and t-test. Results: There were 73 patients identified as having mammographically occult breast cancer and 151 patients had cancers which were mammographically detectable. Relative to patients whose tumors were mammographically detectable, patients with mammographically occult breast cancer had larger tumors (P<0.001), had higher TNM staging (P=0.006), were younger (P<0.001), and were more likely to be ER/PR negative (P= 0.014). There was no significant difference in race (P=0.27), menopausal status (P=.10), nodal status (P=0.90), and HER-2/neu status (P=0.36) between the two groups. Conclusions: These data suggest that tumor size is larger, and subsequently TNM staging is higher in patients who have mammographically occult tumors. However, despite increased primary tumor size, the percentage of patients who presented with node positive disease was not significantly higher. Lastly, mammographically occult cancers may be more likely to be ER/PR negative, which raises the question of whether tumor biology influences radiographic detection.

604 Prevalence and characteristics of radiographically occult breast cancer.

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Background: Increased breast density has been shown to increase the risk of breast cancer. Additionally, lesions in dense breasts are more difficult to detect on mammography or sonography due to obstruction by the breast tissue. In the American College of Obstetricians and Gynecologists (ACOG) management guidelines, there are no formal recommendations on how to manage patients with palpable breast masses which are also radiographically occult.

Materials and Methods: A prospective database was collected from 1999-2002 for all excisional breast biopsies (N=304) from a single breast surgeon. Results: From a total of 304 excisional biopsies, 25 patients presented with negative ultrasound and mammography exams, and all were recommended for excisional biopsy based on the clinical finding of a mass. 8/25 (32%) patients with radiographically occult masses resulted in cancer. Over the same time period, 174 breast cancer patients were also referred for diagnosis and treatment. The radiographically occult patients accounted for 8/174 (5%) of all cancer patients diagnosed. There were no significant differences in tumor characteristics between the occult cancers and non-occult palpable cancers. 7/8 (88%) of the occult cancers had dense breasts according to the American College of Radiology standards, compared to only 50% of women aged 50-60.

Discussion: The finding that 32% of radiographically occult masses are malignant is highly significant. Radiographically occult palpable masses were also more likely to occur in women with dense breasts. Based on these findings, it is recommended that patients with palpable masses that are radiographically occult be referred to a breast specialist who can more accurately determine the need for a biopsy. If evidence of malignancy cannot be established or excluded on clinical grounds, it is recommended that patients have surgical biopsy, or short term follow-up if biopsy is not amenable. This can be expected to result in the biopsy of benign lesions, but removal of them is desirable on other grounds. While in some instances the probability of cancer may be exceedingly small, it is never zero. In this study, the probability of cancer in a radiographically occult lesion was 32% which is deemed to be sufficiently high to warrant biopsy in masses with these characteristics.

605 Establishing a scale of clinically meaningful change in breast density in women using hormone replacement therapy using visual and digital assessment.

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Background: Women with high breast density are at 4-6 fold increase in risk for developing breast cancer. Use of HRT is associated with a small increase in breast cancer risk and is known to increase breast density in 17-73% of women. Combined regimens are associated with higher breast cancer risk and a greater number of women that increase in breast density with use than estrogen alone. Thus, the risk appears to mirror mammographic density and there is increasing interest in the effect of specific agents on breast density. However, while there are several useful standards for assessing breast density at one point in time, no standards exist for defining a clinically meaningful change in breast density.

Materials and Methods: Using our computerized database, we identified 28 cases of postmenopausal women reported to have a change in breast density due to HRT use from 1997-1998. Ten control cases of postmenopausal women with no change in density during the same time period were selected. Mammograms were digitized using a high-resolution Lumisys 75 scanner. Density was visually assessed by one radiologist experienced in breast imaging and classified as 0: No change, +1: Focal or minimal increase in density less than one BIRADS category, +2: Moderate increase in density of one BIRADS category, and +3: Marked increase in density of one or more BIRADS categories with an associated increase in breast size. Digital assessment was performed using segmentation and interactive thresholding to obtain percent density.

Results: Visual assessment resulted in assignment of 9 cases in +1, 10 cases in +2, and 9 cases in +3. Digital assessment of breast density resulted in a mean increase of 6.8% in the +1 group (range 2.0-13.8%), 18.7% in the +2 group (range 13.4-25.2%), and 37.4% in the +3 group (range 25.5-46.6%). The control group had a mean decrease of 1.4% (range +2.3 to -3.5%).

Discussion: Previous studies assessing a change in breast density have used a visual assessment or changes in BI-RADS or Wolfe's categories. Those using visual assessment have not defined the degree of change, which may lead to variability between readers and studies. Use of change in BI-RADS or Wolfe's categories over time is more quantitative, but is a rather coarse assessment of change. In this study, we have defined changes in breast density in clinically meaningful categories and have correlated these with digital assessment of percent change in density. Changes in mammographic density may signify changes in breast cancer risk. These definitions may be useful for quantifying the percentage of women with minimal, moderate, and marked changes in breast density due to different stimulatory or preventive hormonal regimens.

606 Average tumor size and overall survival of patients with primary diagnosis of breast cancer influenced by a more frequent use of mammography.

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Purpose:

Recent meta-analyses give reason to doubt, whether general mammography screening reliably leads to early detection of breast cancer, and subsequently to an improved overall survival. In our study, we investigated, whether the more frequent use of mammography since 1980 has influenced the average tumor size at time of the primary diagnosis and overall survival.

Methods:

From 1981 to 1990, 1656 consecutive patients with primary breast cancer were operated at the I. Frauenklinik der LMU Munich and Frauenklinik Berlin-Charlottenburg, Germany. In a contemporaneous analysis, we compared the average tumor size at the time of primary surgery and overall survival of patients treated during the years 1981-1985 (n=849) and during the years 1986-1990 (n=807), respectively. The mean follow-up was 63 months.

Results:

Both patient groups were comparable in reference to age (p=0,77) and status of axillary lymph nodes (p=0,14). The average tumor size at time of the primary diagnosis continuously decreased during the study period (Pearson's correlation: -0,179, p<0,001). The average tumor size of patients operated until 1985 was 25 mm, compared to 21 mm in patients treated from 1986 on (p<0,001). While until 1985 in 19 % (n=164) of the cases indication for operation was based on mammographical findings, this was the case in 27 % after 1986 (n=215, p<=0,001). Unexpectedly, the reduction of the average tumor size at the time of the primary diagnosis did not lead to an increased overall survival: the median overall survival was 142 months in the first group (CI 95 % 109-118) and 113 months in the later group (137-148, p=0,48).

Conclusion:

The observed decrease of the average tumour size at the time of the primary diagnosis of breast cancer might be caused by a more frequent use of mammography. In line with recent data, this small reduction in tumor size does not necessarily translate into improved prognosis.

607 Use of radiolucent cushion lessens mammography discomfort.

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Objective: For optimal image quality, compression is necessary during mammography. Many women describe discomfort or pain during the imaging process. The breast cancer patient treated with lumpectomy and radiation is more likely to have discomfort with mammography. A radiolucent cushion was examined in these patients to identify value in decreasing pain in this group of patients.

Methods: Twenty-five breast cancer patients were studied during their routine visits in a prospective randomized fashion. Age range of the patients were 46-84 years old. All patients had the same surgeon and radiation therapy facility. Only the irradiated breast was studied. Each patient had two exams of the breast. One exam used standard compression plates with a mammogram exposure. The other exam was exactly the same except the cushion was placed on the compression plates. The patient was positioned for an equal amount of time but without the xray exposure. Patients were randomly assigned to have either the padded exam first or the standard exam first. Questionnaires were filled out before and after the exams. A visual analog scale was used to measure discomfort/pain. Improvement or worsening was measured as a percentage difference from baseline. Results: Overall, 54% of patients documented a decrease in the amount of pain using the visual analog scale with use of the cushion. The degree of pain reduction varied from 25% to 50% in the amount of pain experienced. Generally, the larger amount of pain without the pad, the greater the relief with the pad. Other variables were examined including location of surgery within the breast, time elapsed from surgery, number of lymph nodes removed, and size of primary. There were no variables that correlated with the presence of pain or it's relief with the cushion.

Conclusions: Half of these breast cancer patients found benefit from use of the cushion. This is similar to other initial studies using the pad in non-irradiated patients. This study supports the identification of high-risk groups that might benefit from the use of the cushion.

608 Three-dimensional dynamic MRI (3DMRI) in a supine position can evaluate intraductal spread in breast cancer.

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Background : Breast MRI is becoming more regularly incorporated into clinical evaluations. Patients in general undergo imaging while in a prone position, opposite to the operating position, on a dedicated breast coil.

Purpose : To evaluate the usefulness of 3DMRI, in a supine position using a manufacturer surface coil provided in a general MR unit, for assessing intraductal spread.

Materials and Methods : We undertook preoperative 3DMRI for 116 breast cancer cases and evaluated detailed pathological examinations in 43. During 3DMRI, patients lay supine raising their arms similarly to an operating position. The patients were fixed in an exhaled position by a chest band on the surface coil in order to reduce motion during imaging. Dynamic study was performed and specimens were histopathologically investigated by making serial sections at 5mm width. 3DMRI and histologic results were analyzed for the ratios of detection of intraductal spread, and for the relationships between tumor sizes measured with 3DMRI and those by pathological mapping.

Results: Sensitivity for detecting intraductal spread on 3DMRI was 84% according to pathological mapping. Discrepancies between tumor sizes measured with 3DMRI and by pathological mapping were less than 1cm in 55%, and 2cm in 90%, respectively. Among 74 cases having breast conserving surgery, 21cases(28%) had positive surgical margins.

Conclusion : 3DMRI performed in a supine position could precisely evaluate intraductal spread in breast cancer. Tumor sizes measured with 3DMRI coincided with pathological mapping. We suggest that 3DMRI in a supine position is useful for assessing intraductal spread.

609 MRI measurements of tumor volume and vascularity are valuable prognostic indicators for monitoring neoadjuvant chemotherapy of breast cancer.

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Background: Monitoring response to neoadjuvant chemotherapy can enable us to tailor individual treatments to maximize effectiveness. Previous work has shown that magnetic resonance imaging (MRI) can successfully identify changes in breast tumors with therapy. The purpose of this study was to investigate the predictive value of MR tumor measurements in comparison with clinical variables for predicting disease-free survival and providing early indication of response.

Methods: A group of 52 women undergoing neoadjuvant chemotherapy were imaged before, during (after one cycle), and following completion of treatment. Contrast-enhanced MRI was used to measure the changes in both tumor volume and vascularity based on optimized dynamic enhancement cutoffs. Clinical variables such as patient age, tumor grade, clinical response assessment, and pathology measures were also included in the analysis. The predictive value of MR measurements and clinical variables were then evaluated in comparison with patient outcome.

Results: The parameters which significantly correlated ($p<0.05$) with disease-free survival in univariate analyses were initial and change in tumor volume measured by MRI, and pathology measures of size of residual disease. Proportional hazards survival analysis showed that a model of percent change in MR tumor volume in combination with initial volume had the strongest association with length of disease-free survival and was more predictive than clinical variables. In addition to tumor volume, several vascular parameters measured by MR early in treatment were found to be significantly associated with response markers such as tumor shrinkage, size of residual disease, and number of positive lymph nodes by multivariate stepwise linear regression analysis.

Discussion: These findings indicate that MRI measurement of change in tumor volume is an important predictor of treatment outcome. Interestingly, it was also observed that treatment response may be somewhat dependent on the initial vascular properties of the tumor. This work helps to identify new and valuable markers of treatment response in order to better tailor breast cancer therapies and potentially improve patient outcome.

610 Influence of short-term exemestane treatment on contrast-enhanced (CE) breast MRI: study design and first experiences.

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Background: CE-MRI is a very sensitive method for the detection of malignancy. However, unspecific enhancement of benign tissue can impair assessment by obscuring or mimicking malignancy.

Previously, we showed that short-term anti-estrogen treatment can partially reduce unspecific enhancement. Before such short-term pre-treatment can, however, be considered for diagnostic purposes, it must be excluded that enhancement of malignancy is suppressed. The following study was designed to investigate the effect of short-term Exemestane treatment on contrast enhancement of benign and malignant tissues.

Material and Methods: Patients with a diagnosis of malignancy underwent a study MRI on day 0 and were treated with 25 mg Exemestane until surgery. Thereafter they underwent weekly MRI up to the day of definitive surgery. Enhancement was evaluated visually and quantitatively by 2 experienced readers by agreement.

Results: To date 8 patients have been examined. 2/3 enhancing benign changes regressed. During Exemestane one of 8 tumors appeared slightly smaller. In 2/8 cancers the curve flattered slightly. However, all cancers remained clearly visible.

Discussion: Discrimination of benign and malignant tissues improved. First results are encouraging. More data are being acquired.

611 Application of positron emission tomography in postoperative follow-up of breast cancer.

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Background: Positron Emission Tomography (PET) is an imaging method that can diagnose diseases by detecting physiologic and biochemical changes of the body. In this study, we attempted to evaluate the value of PET in the postoperative follow-up of breast cancer.

Material and Methods: From September, 1994 to December, 2001, 67 cases of postoperative PET were performed at Samsung Medical Center on 66 patients who had curative operation for breast cancer. Clinical outcomes were confirmed by clinical course, fine needle aspiration cytology, biopsy and operation. We reviewed the medical records and reports of PET of these patients.

Results: The time interval between the operation and PET ranged from 1 to 88 months with a median of 26.5months.The reasons for performing PET were abnormal physical examination (23 cases, 34.3%), equivocal result of bone scan (22 cases, 32.8%), other radiologic abnormalities (9 cases, 13.4%), abnormal laboratory findings (4 cases, 6.0%), symptoms of patients (4 cases, 6.0%). Among 67 cases of PET, 48 cases were confirmed as having metastasis or recurrence, while 19 cases did not have metastasis or recurrence. True positive cases were 45 cases, true negative cases were 16 cases, false positive cases were 3 cases and false negative cases also were 3 cases. Therefore, the value of PET in the detection of postoperative metastasis or recurrence of breast cancer was 93.8% in sensitivity, 84.3% in specificity, 93.8% in positive predictive value, 84.3% in negative predictive value and 91.0% in accuracy. On the basis of the lesion site, the accuracy of PET in the detection of bone (98.5%), lung (100%) and liver (98.5%) metastasis was superior to that of local recurrence (85.1%) or lymph node metastasis (86.6%).

Discussion: PET may be helpful in a selected subgroup of patients for whom findings are inconclusive after conventional postoperative follow-up method of the breast cancer, especially in the detection of bone, lung, and liver metastasis.

612 A comparison study of ^{18}F-labeled choline versus ^{18}F-labeled 2-deoxyglucose positron emission tomography for evaluation of advanced breast cancer.

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Background: The role of positron emission tomography (PET) in breast cancer evaluation is expanding. To date, PET imaging has predominantly employed the glucose analogue ^{18}F fluoro-2-deoxyglucose (FDG). However, FDG-PET has been found to have less sensitivity and/or specificity for assessment of some types of cancer, and has shown limited results for locoregional evaluation of early stage breast cancer. ^{18}F fluorocholine (FCH) is a novel PET probe capable of imaging the increased choline metabolism identified in breast malignancies. **Materials and Methods:** 24 patients with locally advanced or metastatic breast cancer were studied by FDG and FCH PETon separate days prior to initial treatment, or upon disease progression. Conventional staging studies were performed concurrently, and data on hormone receptor (HR) status were also collected. Standardized uptake values (SUV) were measured for identified lesions and compared by site of involvement and hormone receptor status. **Results:** 57 lesions were identified by FCH and/or FDG PET. There was a general pattern of higher SUV's for FCH in HR+ lesions. The average FCH SUV was 2.6+/-1.2 for HR- lesions and 4.3+/-2.8 for HR+ lesions ($p<0.05$); no significant difference was seen for FDG uptake by HR status. Moreover, for HR+ lesions five were discordantly FCH positive/FDG negative, with only one being FCH negative/FDG positive. Liver lesions were less well defined by FCH PET because of normal liver uptake; however, lack of cardiac uptake facilitated identification of intrathoracic lymph nodes. **Conclusions:** FCH is a promising probe for PET breast cancer imaging. Choline metabolism may be increased in HR+ lesions, perhaps making it a better probe for this type of breast cancer. In addition, it provides clearer thoracic imaging, warranting further investigation of its use in evaluating locoregional disease.

613 The full potential of breast cancer screening has not yet been realized in the United States.

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Background: Mammography use has increased dramatically in the past two decades among women 50-69 years of age in both the United States and in western European countries. It has been suggested that the reduction in breast cancer mortality among women with screen-detected cancers results from detection of tumors with smaller mass, in an earlier, less aggressive state, and before they metastasize to lymph nodes. The purpose of this study was to determine if the increasing use of mammography over time affected changes in early indicators of the effectiveness of screening that precipitated the reduction in breast cancer mortality that has been observed in the United States.

Material and methods: The analyses are based on women 50-69 years of age diagnosed with primary breast cancer using data from the Surveillance, Epidemiology, and End Results (SEER) program (1990-1998). Five indicators of the effectiveness of breast cancer screening that are precursors to reductions in mortality are described: in situ breast cancer; stage II+ tumors; lymph node-positive cancers; and T3/T4 breast cancer.

Results: The in situ rate increased from 37.8 in 1990 to 67.0 per 100,000 population in 1998. The rate of T1 tumors (< 2 cm) increased slightly over time from 143.5 in 1990 to 163.5 per 100,000 in 1998; the rate of T2 tumors (2-5 cm) remained unchanged. Average annual rates were stable at 10.0 per 100,000 for T3 tumors and 7.1 per100,000 for T4 tumors during this time period. The rate of stage II+ cancers and those that were node positive remained also stable at about 120 and 76 per 100,000 population, respectively.

Discussion: The lack of a decline in the rate of LABC and node-positive cancers over time among the nine SEER registries suggests a lack of widespread routine screening adoption despite continuing increasing screening prevalence in the United States. Although screening results in predictable patterns of disease and despite the effectiveness of screening to reduce mortality from breast cancer, the decline in breast cancer mortality in the United States during the 1990s is not likely due to the influence of screening.

614 Palpation of the supine patient is the necessary step for a screening clinical breast examination.

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Over one-third of breast cancers are first detected by palpation (often by the woman herself). However, clinical breast examination (CBE) is often omitted in primary care practice, presumably in part because of time required for the multiple steps recommended for routine CBE (Kottke, Mayo Clin Proc 1997; 72: 515). Mahoney and Csima (Can Med Assoc J 1982; 127: 729) suggested simplification of routine CBE to palpation of the patient when she is supine - without visual inspection while sitting and/ or during arm maneuvers.

METHODS: To determine whether omission of visual inspection would miss cancers, we reviewed the recorded CBE of 487 consecutive breast cancers of any stage. Our CBE palpation method indexed ability to feel rib edges through breast tissue as an aide to identify subtle changes.

RESULTS: 316 cancers had a positive CBE (232 a mass; 75 subtle changes, i.e. asymmetry or vague density; 9 visible changes only). 392 had a positive mammogram. 8 cancers were missed both by palpation and by mammograms. 4 had negative mammograms and only visible changes on CBE (2 Paget's disease also visible when supine, one with a large axillary node, ONLY ONE with ONLY skin retraction). Four cancers with both negative mammogram and negative CBE were found only in the pathology specimen from a prophylactic mastectomy.

CONCLUSIONS: Palpation of the supine patient as the only CBE - combined with mammography - detects over 99% of breast cancers. It is an ethical question whether to simplify recommendations for CBE - with the intent to increase overall frequency of CBE by primary care physicians - while knowing that rarely a cancer will be missed. However, only one of 483 detectable cancers would have been missed if routine CBE were simplified to supine palpation alone. Our results support modification of recommended routine screening CBE to the single step of palpation of the supine patient.

615 Was there a post-randomization bias in the HIP breast cancer screening trial?

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Introduction: The Health Insurance Plan (HIP) of Greater New York initiated a randomized clinical trial in 1963 to determine if screening for breast cancer with mammography and clinical exam would decrease breast cancer mortality. This trial served as a landmark study, demonstrating a mortality reduction, promoting subsequent trials and eventually contributing to the adoption of a screening policy in the US and abroad. Recently, the HIP study was criticized as having post-randomization bias that favored screening by creating a control group with more women who had a history of breast cancer than the study group. Our aim was to re-analyze the HIP survival data for this post-randomization bias.

Methods: We compared survival curves of HIP breast cancer patients who were identified in the "pre"-equalization and "post"-equalization time periods. The equalization time of the HIP trial is defined as the time from trial entry when the number of breast cancer cases is similar between the study and control groups. Most prior analyses of HIP included only breast cancer patients identified in the pre-equalization period in order to avoid diluting the impact of screening, given that the study was designed as a "stop-screen" trial. We tested the hypothesis that the survival curves measured from entry (and diagnosis) of the control groups pre- and post-equalization would be similar, assuming a post-randomization bias did not exist. To ensure that we were comparing similar groups, we matched the range of ages at entry to the pre- and post-equalization periods, and the time allowed for the accrual of breast cancer cases and follow-up.

Results: Survival curves from time of entry (or diagnosis) for the control group pre- and post-equalization show statistically significant differences ($p<0.05$). Pre- and post-equalization survival curves from entry for the study group show similarities to the post-equalization control group. Analyses by age-groups led to less consistent results, which may be due to sample sizes variations.

Conclusion: The post-equalization control group experienced better survival outcomes than the pre-equalization control group. One possible explanation could be a post-randomization bias. Despite this conclusion, we support the position that screening practices today have undergone major advances compared to those of the 1960's.

616 Family physicians' awareness of risk assessment for young women at risk of developing breast cancer.

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Background: Some women are at high risk of developing breast cancer (BC) at a young age, including those with extensive family histories and BRCA1/ 2 mutations. These women represent a unique population for which there is little information with regards to appropriate screening and assessment of risk factors. Such women often seek advice from family physicians (FP) regarding risk assessment and diagnostic screening.

Purpose: The purpose of this study was to determine FP level of awareness of risk assessment in this population as well as to identify if additional educational resources would be beneficial.

Methods: Family physicians from Northwestern Ontario completed a survey containing 11 statements pertaining to their knowledge of monitoring procedures and risk assessment tools available for young women at risk of developing BC. Responses were recorded using a visual analogue scale (7 questions) and categorical responses (4 questions).

Results: A total of 74/179 (41.3%) of FP responded to this survey, mailed in February, 2001. The majority of FP (81%) felt satisfied with their current level of knowledge of risk factors associated with the development of BC. Most FP (73 %) felt that baseline mammograms should be performed yearly in high risk women starting at age ≤40 (57% FP). Mammogram/breast ultrasound (76%) and clinical exam (82%) were felt to be the most appropriate screening tools for women presenting with breast symptoms. The majority (73%) felt satisfied with screening resources available for younger women and almost all FP (91%) actively monitor this population. Genetic counselling was not available in the majority of communities (62%), although FP were aware (86 %) of where to refer women. Almost half (46%) felt that further education for FP was needed to adequately assess these women. Lectures (88%) and written literature (76%) were felt to be the best educational tools.

Discussion: The majority of FP actively monitor young women at risk of developing BC. A written assessment tool is currently being developed for FP.

617 Targeted interventions can improve rescreen compliance in a regional mammographic screening program.

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Background: Effective mammographic screening requires a high compliance for continued rescreening. In Australia the rescreen interval is two years, and women are sent a reminder letter when their rescreen is due. Poor compliance progressively reduces the number of women attending for screening at later rounds. A constant rescreen rate of 80% for each subsequent screening round would leave only 33% of the original prevalent screen population after five screening rounds. Failure to respond to two rescreen reminder letters, incorrect addresses and failure to attend after an appointment is made (DNA), all contribute to lower rescreen compliance, and the latter wastes resources. Our aim was to determine whether additional targeted interventions could improve rescreen compliance.

Methods: Three (3) interventions were tested: (i) Rescreen non complier telephone calls to women who had not responded to two rescreen reminder letters. (ii) For rescreen reminder letters returned as "not known at this address", the woman's family doctor was telephoned to seek an updated address. Rescreen reminder letters were then sent to the new address. (iii) A pilot randomised trial compared telephone call, letter, both, or neither as a "prompt" after a rescreen booking was made, to assess feasibility, and impact on DNA rates.

Results: (i) Of 3,372 women not responding to two rescreen reminder letters over 24 months, contact was made by telephone with 1,913 women, of whom 1,504 (48.6%) made and kept an appointment. The overall re-attendance of women who received the telephone reminder was significantly higher than that of women who had not received this prompt (78.6% vs 40.8%, p = < 0.0001). Of unsuccessful contacts, 31.5% were found to be "left address/ wrong number". (ii) Of 1,154 rescreen reminder letters returned, 543 women had a new address identified following family doctor telephone calls, and 329 of these women completed their screening appointment. Significantly more women for whom a new address was obtained, made rescreen appointments, than did those without an updated address (60.6% vs 4.1%, p = < 0.0001). (iii) DNA rates for women with rescreen bookings were reduced from 7.4% (28/379, controls) to 4.2% (47/1123, interventions) (p < 0.01). Intervention with a prompt (telephone, letter, or both) significantly reduced the DNA rate. There was no significant difference between the different interventions. Costs were minimal.

Conclusion: Targeted interventions, (rescreen reminder calls, correction of addresses, and prompts for women with booked rescreen appointments), can all improve rescreen compliance. Average rescreen compliance rates were 93.2% for 2001.

618 Comprehensive screening using breast MRI and ductal lavage in high-risk women.

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Background: Women who carry BRCA1/2 mutations have a 50-85% lifetime risk of developing breast cancer. Standard of care for breast cancer risk management in these patients include surveillance or prophylactic mastectomy (PM). Surveillance, consisting of clinical breast exam (CBE), and mammography is recommended despite the unproven mortality benefit. We have initiated a comprehensive screening protocol including breast MRI and ductal lavage (DL) in the hopes of identifying early breast cancer or premalignant lesions as an alternative to standard surveillance and PM. Breast MRI promises to increase the rate of early detection and DL to improve risk assessment. Breast MRI screening has shown superior sensitivity to mammography in 6 screening pilot trials, yet there are concerns of low specificity resulting in additional procedures and compromising quality of life. The combination of atypia detected by DL and a family history of breast cancer increases risk 11-22%.**Methods**: Eligibility criteria included either known BRCA1/2 mutation carriers or women with a 15% risk of developing breast cancer at 10 years. Our 1.5 Tesla breast MRI protocol produces rapid dynamic images of contrast enhancement using 3D spiral MRI and high spatial resolution images of lesion morphology during peak enhancement using centric 3DSSMT. DL was conducted on all fluid-yielding, and when possible, 1 non-fluid yielding duct in each breast. Patients had to have a negative CBE and mammogram. **Results**: We have screened 18 women with MRI, 9 of whom have had DL. Seventeen women have undergone genetic testing, of which 7 are BRCA1 carriers and 3 are BRCA2 carriers. Seven patients had abnormal findings on MRI based on enhancement patterns and morphology and underwent biopsy. Two patients had an abnormal pathologic diagnosis; 1 patient had high-grade DCIS and the other had a radial scar. Both patients were BRCA1 carriers. The remaining 5 patients had benign findings. We have identified 1 case of atypia on DL in a patient with a normal mammogram and MRI. After one round of screening, 2 women have opted to have PM. **Discussion**: Our comprehensive screening protocol promises to identify early cancers and pre-malignant lesions in high-risk women and may provide information about risk assessment to help guide women's choices about risk management.

619 Vitamin E acetate supplementation may be detrimental to tamoxifen efficacy.

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BACKGROUND: Cancer patients frequently take vitamin E supplements during therapy despite a lack of evidence of benefit. In addition to inhibition of the estrogen receptor (ER), there are a variety of non-ER pathways that are thought to contribute to the antitumor effect of tamoxifen. The in vitro induction of apoptosis by tamoxifen has been postulated to involve oxidative stress. Our hypothesis that supplemental vitamin E (alpha tocopherol acetate), alters the effectiveness of tamoxifen was tested using in vitro and in vivo models of human breast carcinoma.

MATERIAL AND METHODS: The ER-positive breast cancer cell line MCF-7 was cultured in a series of concentrations of alpha tocopherol (AT) alone and in combination with 20 µM tamoxifen (TAM). Proliferation was determined by MTS assay. The in vivo effect of AT on tamoxifen efficacy was studied on established MCF-7 xenografts in female nude mice. Six week-old female nude mice were supplemented with estrogen by subcutaneous pellet and received 4 million MCF 7 cells injected in the mammary fat pad. After 7 days, TAM 1mg/kg was administered daily to all mice by ip injection. The mice were randomized into 2 treatment groups; one group receiving regular chow, one group receiving AT 1.65 g/kg/day in chow. Breast tumor growth was measured by caliper twice a week and determined by the mean of 2 diameters. RESULTS: TAM decreased in vitro proliferation of MCF-7 by 80%. AT in concentrations of 10-800 µM increased MCF-7 proliferation compared to media alone. The addition of AT at all concentrations to TAM caused a 3-fold increase in proliferation of MCF-7 compared to TAM alone (p<.05). The mean tumor diameter in the AT chow group (15.8 mm) was significantly greater than the regular chow group (10.9 mm). CONCLUSION: These studies suggest that alpha tocopherol decreases the efficacy of tamoxifen. Because the mechanism of action of certain therapies involve the generation of oxygen radicals, it is an item of debate whether antioxidant supplements should be avoided. We alert the public and the medical community to an unintended interference by combining vitamin E alpha tocopherol with tamoxifen in the adjuvant treatment of breast cancer.

620 The effect of phytooestrogen ingestion on vasomotor menopausal symptoms.

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Background: Vasomotor menopausal symptoms lead to considerable morbidity in the western world. A high dietary intake of phytooestrogens is associated with prolongation of the menstrual cycle, reduction in the incidence of breast cancer in Asian women and a decrease in menopausal symptoms.

Methods: A randomised placebo-controlled crossover trial was conducted to examine the effect of dietary supplementation with lignans on menopausal hot flushes. Postmenopausal women suffering at least five hot flushes / night sweats per 24 hours were randomised to either 40 grams of flaxseed food supplements, or placebo, per day for three months. All subjects acted as their own control and crossed-over. Hot flushes were counted daily during the six months of the trial. Luteinising hormone (LH), follicle stimulating hormone (FSH), prolactin, growth hormone (GH), IGF-1, and cholesterol were measured in the serum.

Results:

Table 1.

	Prolactin (mu/l)	IGF-1 (mu/l)	Median hot flushes
Baseline	179.7	20.6	206
Post-placebo	191.4	21.9	146
Post-flaxseed	208.2	22.5	59* *p<0.001

Statistics: two sample t-test and Mann-Whitney U test

An increase in prolactin and IGF-1 were observed on post-flaxseed diet but not significantly. Comparing the changes in the number of hot flushes between baseline, three and six months we found a statistically significant reduction in the number of hot flushes on flaxseed supplementation, p=0.001 (Mann-Whitney U test).

Discussion: Phytooestrogens such as lignans could potentially be used as an alternative for hot flushes in women who have undergone breast cancer treatment.

621 Cimicifuga racemosa has no growth-stimulatory effect on the estrogen-dependent human breast cancer MCF-7 in nude mice.

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Clinical studies have shown that Cimicifuga racemosa (CR) alleviates menopausal symptoms. It has, however, not been investigated whether CR stimulates the growth of breast cancer.

The purpose of the present study was to investigate the possible effect of CR on estrogen-dependent MCF-7 tumors grown in nude mice.

CR from Angelika, (Ferrosan A/S, Denmark) was administered orally and the effect on uterine wet weight and on the growth of the estrogen receptor positive (ER+) MCF-7 and the ER- MDA-MB-231 human breast cancer xenografts was investigated.

The results showed that untreated controls and mice receiving CR (2.4 mg/kg - 240 mg/kg)did not develop MCF-7 tumors, whereas estrogen supplementation (0.72 mg slow release pellets) of the ovariectomized tumor inoculated mice had the expected tumor growth stimulatory effect.

CR had neither stimulatory nor inhibitory effects on MCF-7 tumor growth when combined with estrogen.

Growth of the ER- MDA-MB-231 was not affected by CR (2.4 mg/kg) treatment.

An increase in uterine wet weight in CR (2.4 mg/kg) treated mice corresponding to that obtained by estrogen, suggest that CR has estrogenic potential in normal murine target tissue.

These results suggest that CR contains phytoestrogens with potential estrogenic effect on normal target tissues but without growth stimulatory effect on breast cancer.

622 NAT2 interactions on breast cancer risk: SULT1A1 and cooked meat.

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Background: Heterocyclic amines (HA) and polycyclic aromatic hydrocarbons(PAH) are two major classes of carcinogens generated by cooking meat at high temperature. HA are metabolized by sulfotransferase 1A1 (SULT1A1) and N-acetylation, which is governed by NAT 1 & 2. SULT1A1 is also involved in the metabolism of estrogen. We present findings on associations between cooked meat consumption, polymorphisms in NAT2 and SULT1A1, and breast cancer risk.

Materials and Methods: A hospital based case-control study was conducted at the New York-Presbyterian Medical Center (NYPMC). Data were available on 103 cases, 2 control groups, one comprised of 97 women with benign breast disease and the other comprised of 133 women visiting NYPMC for routine gynecological checkups (healthy controls). Blood samples were collected from cases and controls. All subjects were interviewed regarding known breast cancer risk factors, occupational and environmental exposures, smoking history; alcohol consumption; family history of cancer and dietary exposure to "cooked meat" carcinogens (from broiling, frying and BBQ cooking of meat in the past 2 weeks). NAT2 (slow/fast) and SULT1A1 (His/Arg) polymorphism at codon 213 was determined by PCR RFLP analysis using DNA from white blood cells.

Results: Subjects reported an average of 5 servings of "cooked meat" in the past 2 weeks. Only in the NAT2 slow subjects, "cooked meat" was associated with increased risk of breast cancer (BC) (p=0.038). All analyses used healthy controls. BC risk for 5 servings of "cooked meat" was 2.12, p=0.005 adjusting for known risk factors. There was an interaction between NAT2 and cooked meat (dichotomized) adjusted for known risk factors (p= .043). SULT1A1 alone was associated with BC risk (OR= 1.5, p=0.052). The risk was limited to NAT2 slow subjects (OR=1.9 p=0.045) vs. rapid subjects (OR= 0.95, p = 0.88) adjusted for known BC risk factors and cooked meat. In that model the OR for 5 servings was 1.7, p=0.025 in the NAT2 slow strata. The interaction between NAT2, SULT1A1 (arg/his or his/his) genotype and BC status was p=0.042.

Discussion: NAT2 alone did not predict BC risk. However, this analysis found a gene-environment interaction between NAT2 and "cooked meat", and a gene-gene interaction between NAT2 and SULT1A1 and breast cancer risk. Previous analyses within this study found a significant interaction between NAT2 and alcohol consumption with highest risk among alcohol consumers who were NAT2 fast. The lack of consensus regarding NAT2 and BC risk may be due to its involvement in the metabolism of multiple substrates and possible gene-environment and gene-gene interactions.

623 Comparison of random periareolar FNA cytology in high-risk NAF producers versus non-NAF producers.

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Evidence of hyperplasia with atypia found by random periareolar fine needle aspiration (FNA) and production of nipple aspirate fluid (NAF) are both associated with increased risk for breast cancer (Fabian 2000; Wrensch 2001). In this study, we attempt to determine if NAF production is associated with evidence of hyperplasia with atypia found by FNA. We present data collected from high-risk women attending the Breast Cancer Prevention Center at the University of Kansas Medical Center. These seventy-one women underwent random periareolar FNA and attempted NAF production. Forty-two percent of the NAF collection attempts occurred on the same day as the FNA procedure, 21% of the attempted NAF collections occurred prior to the date of the FNA and 37% of the attempted NAF collections occurred after the date of the FNA, but all occurred within a 12-month period. The median age of the 71 subjects was 46, the median 10-year Gail risk assessment was 6.0%, and 51% were postmenopausal. NAF was successfully collected in 59% of the 71 subjects. Between NAF producers and non-producers, there was no significant difference in median age (46 vs. 46), post-menopausal status (50% vs. 52%), hormone use (50% vs. 47%), positive family history of breast cancer (67% vs. 66%), or 10-year Gail risk assessment (6.3% vs. 5.6%). Twenty-one of the 71 subjects were found to have hyperplasia with atypia by random periareolar FNA. There was no significant difference in the prevalence of FNA atypia for those who produced NAF (14/42) vs. the non-NAF producers (7/29) p=.69. Although production of NAF has been reported to be associated with increased breast cancer risk , failure to produce NAF does not exclude the presence of hyperplasia with atypia by random fine needle aspiration.

624 The Gail model deemed inappropriate for the Czech population. Results of case-control study in 4188 women.

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The Gail model has been developed to identify high-risk women for developing breast cancer. This model is widely used in the United States. The validity of Gail model has not been confirmed in different populations throughout the world. The national case-control study to answer this question has been performed in the Czech Republic.

Methods: Between the year 2000 and 2002, 14 327 questionnaires containing patient's history data were collected. The cases consisted of women, who were diagnosed with breast between the year 1985 and 2002. For each case, an age-matched control was selected from women who underwent regular screening mammography. Total number of age-matched case-control pairs was 2094 that means 4188 women. The baseline risk for the Czech population was recalculated according to data obtained from National Cancer Registry. Results: The baseline risk estimated for white American women is approximately 1.5 higher than for Czech women. The baseline curves in the Czech Republic and the US are non-proportional. The relative risk of age at menarche, age at first live birth, the number of previous biopsies and the number of first-degree relatives with breast cancer differ significantly in the Czech population in comparison to study group calculated in the original Gail model. New coefficients have been used to describe the risk of breast cancer in every individual woman. Conclusion: The original Gail model is not appropriate for the Czech population. Recalculation of relative risks allows physicians to implement modified version of Gail model in medical counseling in breast cancer prevention in the Czech Republic.

625 Association of Cyp19 (aromatase) and SHBG gene polymorphisms with plasma hormone levels in postmenopausal women: implications for breast cancer.

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It is likely that there are multiple low-penetrance breast cancer (BC) susceptibility genes but their identification is difficult. One approach is to assess the relationship between bi-allelic genetic polymorphisms (SNPs) and established risk factors for BC. We have studied the association between plasma levels of hormones known to be associated with BC risk: oestradiol (E2), testosterone (T), oestrone, oestrone sulphate, androstenedione, 17-hydroxyprogesterone, & sex hormone binding globulin (SHBG) and SNPs in genes involved in their synthesis or control (COMT [4 SNPs], CYP17 [3], CYP1B1 [3], SHBG [2] & CYP19 [2]), Hormone profiles were measured in single plasma samples from 1800 normal postmenopausal women not taking HRT from East Anglia, UK, involved in a European cohort study of diet and cancer (EPIC). SNP genotyping was carried out on lymphocyte DNA using Taqman™ (Applied Biosystems). A g-a SNP in the 5'UTR of SHBG gene was significantly associated with both SHBG levels ($p<10^{-5}$) and E2:SHBG ratio ($p<10^{-3}$). Carriers of the a allele had dominantly increased SHBG levels (means: gg [n = 819] 39.5 nmol/L, ga [663] 44.1 nmol/L & aa [255] 43.4 nmol/L) and reduced E2:SHBG ratio. In addition a silent t-c SNP in 3'UTR of Cyp19 was significantly associated with E2 level ($p< 10^{-4}$)and E2:T ratio ($p<10^{-7}$,means: tt [n = 479] 0.0259, tc [875] 0.0234 & cc [393] 0.0209). The same t allele has previously been reported to be associated with raised Cyp19 mRNA levels in tumours (Kristensen et al. Oncogene: 19; 1329-33, 2000). However, it is not yet known if these SNPs are themselves functional or markers for other, as yet undiscovered, functional variants. These data provide evidence for a genetic contribution to the controls of hormone parameters related to BC risk.

(Supported by Cancer Research UK).

626 Substantial variation of NAF estrone and estradiol levels between ducts in high- risk women.

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Nipple aspirate fluid (NAF) production, use of hormone replacement therapy (HRT), and serum levels of estradiol (E2), have all been associated with increased risk for breast cancer. NAF production is influenced by multiple factors including systemic and local hormone levels. It has previously been reported that NAF estradiol (E2) and estrone (E1) levels are higher than serum, possibly as a result of local aromatase and sulfatase activity (Ernster 1987). We examined the possibility that estrogen levels might differ between ducts within the same woman by measuring NAF and serum E2 and E1 levels. NAF collection was attempted on 104 high-risk women attending the Breast Cancer Prevention Center at the University of Kansas Medical Center and 63 women produced NAF from at least 1 duct. Of these 63 NAF producers, 31 women produced NAF from multiple ducts (total of 90 ducts) at the same aspiration setting. NAF and serum E2 and E1 values are available on 29 of the 31 subjects. Fluid from each NAF producing duct was collected separately, samples were diluted with water, and E2 and E1 levels were assayed by radioactive immunoassay (RIA). The median number of fluid yielding ducts was 3 [range 2-6] and the median NAF volume was ~3ul.(Table 1). Serum was obtained at the time of the NAF collection and analyzed by RIA.

Median NAF E2 levels were 135x that of serum for pre-menopausal women, 101x for post-menopausal women not on HRT, and 72x for post-menopausal women on HRT. Median NAF E1 levels were 330x that of serum for pre-menopausal women, 632x for post-menopausal women not on HRT, and 600x for post-menopausal women on HRT.

Our preliminary observations indicate marked variation in NAF E2 and E1 concentrations between two or more ducts in the same woman.

Table 1: Comparison of NAF E1 & E2 concentrations of highest to lowest values from separate ducts within the same woman

	Median Age	Median 10yr Gail	Median NAF E2 concentration pg/ml	Median NAF E1 concentration pg/ml	E2 (fold difference)	E1 (fold difference)
Post Menopausal-HRT n=7 [range]	51	6.8%	6645 [380-51,901]	15379 [9885-97,462]	2.5x [1-135]	5x [1-34]
Post-menopausal-no HRT n=9 [range]	46	7.2%	11960 [3148-29,023]	20390 [3090-26,260]	17.5x [7-26]	3.5x [2-31]
Pre-menopausal n=15 [range]	40	4.6%	7802 [2819-27,972]	11599 [980-36256]	3.5x [1-600]	2.5x [1-284]

627 Imbalance of estrogen homeostasis in human breast carcinomas: possible biomarkers for susceptibility to breast cancer.

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Background: Exposure to estrogens has been associated with increased risk of developing breast cancer. We hypothesize that certain endogenous estrogen metabolites, catechol estrogen-3,4-quinones, can react with DNA to cause specific damage leading to the initiation of breast cancer.

Materials and Methods: To investigate this hypothesis, extracts from breast biopsy tissues were analyzed for 31 estrogen metabolites and catechol estrogen quinone-glutathione conjugates. Approximately 1-g specimens of "normal" breast tissue from 49 women without breast cancer (controls) and 28 with breast carcinoma (cases) were analyzed by HPLC with electrochemical detection.

Results: The levels of estrone and estradiol were higher in the cases than in the controls. As expected, more 2-catechol estrogen (2-CE) than 4-CE was observed in controls. The 4-CE were 3.5 times higher in cases than 2-CE and were 4 times higher than in controls. Less methylation was observed for the CE in cases than in controls. The level of catechol estrogen quinone conjugates in cases was 3 times that in controls, suggesting a higher probability for the quinones to react with DNA in the cases. The levels of estrogens and quinone conjugates were highly significant predictors of breast cancer, while the levels of methylated CE were significant predictors of protection against breast cancer.

Discussion: These results support the hypothesis that catechol estrogen-3,4-quinones can damage DNA to initiate breast cancer and suggest that some catechol estrogen metabolites and conjugates can serve as biomarkers to predict risk of developing breast cancer.

Supported by grants P01 CA49210 and R01 CA49917 from the National Cancer Institute).

628 Leptin expression in breast nipple aspirate fluid (NAF) is influenced by body mass index (BMI) and menopausal status.

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Background: The link between obesity and breast cancer has been postulated but the molecular mechanisms involved are unknown. Leptin, the product of the ob (obesity) gene, is a cytokine which controls food intake and energy balance. The expression of leptin in serum is higher in women than in men and is increased in individuals with high (over 25) BMI. Leptin has recently been implicated in the growth of breast cancer cells in vitro. We hypothesized that leptin was present in NAF, indicating that it is secreted by and may interact with breast epithelial cells. We assessed the associations between NAF and in serum leptin levels, and between NAF leptin and BMI.

Materials and Methods: 70 NAF specimens from 62 subjects and 14 matched serum specimens were collected after informed consent and evaluated for leptin expression using an anti-human leptin ELISA kit (Linco Research, St. Charles, MO). Spearman's correlation coefficients were calculated for the association between leptin expression and BMI (Table).

Results: Neither NAF (7.2 vs. 7.6 ng/ml) nor serum (27.8 vs. 24.5 ng/ml) mean leptin expression differed by menopausal status. NAF and serum leptin were associated in both pre- (r=.71) and postmenopausal (r=.37) women. NAF leptin was associated with BMI in pre- but not in postmenopausal women (Table), and the association was stronger after controlling for NAF total protein (tp).

Association of NAF Leptin Expression with BMI

	N	r (tp control)	P value(tp control)
Overall	70	.23(.29)	.060(.016)
Premenopausal	28	.44(.49)	.019(.009)
Postmenopausal	42	.083(.17)	.60(.29)

Discussion: There is ongoing debate over the role of leptin in breast cancer. Our findings indicate that 1) leptin is present in the breast and secreted into NAF, 2) levels in premenopausal women are associated with BMI, a known risk factor for breast cancer, and 3) controlling for tp in NAF samples is worthwhile.

629 Determinants of cathepsin D, a marker of estrogen action, in nipple aspirate fluid.

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Background: Cathepsin D (CD) synthesis is increased by classical estrogen action, and an increase in CD levels has been associated with the neoplastic process in the breast. We studied the biochemical factors associated with CD levels in nipple aspirate fluid (NAF).

Materials and Methods: Subjects were 16 normal premenopausal women sampled 4 times over 12 months. NAF and blood samples were collected at midluteal phase. NAF was diluted with PBS and extracted with ethyl acetate-hexane (3:2). The aqueous phase was assayed for CD, EGF, IL-6, and estrone sulfate (ES). The organic phase was assayed for estradiol (E2). Serum was also assayed for E2 by RIA..

Results: CD levels in NAF ranged from 1 to 16 ug/ml (0.1 to 3 ug/mg protein). Periodic elevations of CD occurred in one or the other breast in most women. Within-woman CD levels between breasts were not correlated (r = 0.05). By stepwise ANOVA, the main components for estimation of CD were EGF and ES with standard coefficients of -0.41 and 0.30 (p = 0.001 and 0.01), respectively. Serum E2 and NAF IL-6 had standard coefficients of only 0.05 and 0.09 (p = 0.59 and 0.31), respectively. Too few NAF E2 data were available for this analysis.

Discussion: The wide differences in CD between breasts is similar to our earlier results with NAF E2. IL-6 promotes aromatase activity but was not associated with CD in this group. NAF ES is important for production of E2 in the breast and presumably is acting to stimulate CD production through conversion to E2. The strong negative association of EGF with CD was unexpected and remains to be explained. We conclude that CD is regulated to a large extent by factors produced locally in the breast. Among these factors are estrogens and EGF.

(Supported by NIH grants P50 CA89018-01 and NCRR M01 RR00048).

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MOVED

631 Does lobular carcinoma *in situ* increase the risk of recurrence in patients with invasive lobular carcinoma?

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Background: Lobular carcinoma *in situ* (LCIS) increases a patient's risk of developing invasive mammary carcinoma in either breast, but does LCIS remain a risk factor after the diagnosis of invasive lobular carcinoma (ILC)? We hypothesized that patients with ILC+LCIS were at greater risk for a less favorable outcome than those with ILC-alone.

Materials and Methods: We reviewed the records of patients at our breast center to identify those whose ILC was treated by breast conservation or mastectomy and sentinel lymphadenectomy between October 1991 and May 2001. Presentation, presence of LCIS, tumor characteristics, prognostic factors and surgical treatment were analyzed for statistical significance.

Results: There were 103 cases of ILC in 102 patients. Demographics for the ILC+LCIS group are compared to the ILC-alone group (Table 1). There were no differences in histologic differentiation, angiolymphatic invasion, DNA ploidy, S-phase, or receptor status between the groups. The ipsilateral recurrence and/or contralateral new primary rate was 4.2% in the ILC+LCIS group at a mean follow up of 47 months and 3.1% in the ILC-alone group at a mean follow-up of 37 months.

Table 1

	ILC+LCIS (%)	ILC-alone (%)	P-value
Number of cases	71 (69)	32 (31)	
Mean age	58.4 + 10.5	67.0 + 13.3	0.0006
Mean T (cm)	2.77 + 2.0	3.25 + 2.35	0.2899
Mean T1 (cm)	1.10 + 0.39	1.35 + 0.47	0.0672
Mean T2 (cm)	2.97 + 0.69	3.74 + 1.02	0.0106
Mean T3 (cm)	6.45 + 1.18	7.13 + 1.56	0.3131
Breast conservation	55 (77)	26 (81)	0.664
Sentinel node-positive	36 (51)	15 (47)	0.719

Discussion: ILC is frequently associated with LCIS, particularly in younger patients with smaller tumors. However, the association of LCIS with ILC does not seem to be correlated with a greater subsequent risk for developing an ipsilateral or contralateral breast cancer when compared to ILC without associated LCIS.

632 ATAC ('Arimidex', Tamoxifen, Alone or in Combination) trial–anastrozole is superior to tamoxifen as adjuvant treatment in postmenopausal women with early breast cancer.

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In a randomized, double-blind design, the ATAC trial compared adjuvant treatment with tamoxifen (T) (20 mg), 'Arimidex' (anastrozole [A], 1 mg) or the combination of A plus T (C). Postmenopausal (PM) patients with operable early breast cancer (BC) were randomized following surgery +/- chemotherapy +/- radiotherapy. Main endpoints were disease-free survival (DFS) and tolerability. Other endpoints included time to recurrence (TTR: censoring non-BC deaths before recurrence) and the incidence of contralateral (CL) BC. A total of 9366 patients were recruited (N=3125, 3116 and 3125 for A, T and C, respectively). Median duration of therapy was 30.7 months and median follow-up was 33.3 months. Total events were 317, 379 and 383 for A, T and C, respectively. 84% of patients were known to be ER+ and/or PR+. As previously reported, DFS was significantly improved in the overall population for A vs T (HR=0.83, 95% CI [0.71-0.96], p=0.013). We now report detailed information on TTR. Greater improvements were seen for TTR with A vs T (HR=0.79, [0.67-0.94], p=0.008). Starting from Year 2, a difference emerged in the annual BC event rates between A vs T and A vs C in favor of A (table).

Year	Annual BC event rates (%)			Hazard ratio		
	A (n=3125)	T (n=3116)	C (n=3125)	A/T	C/T	A/C
1	2.49	2.30	2.82	1.08	1.23	0.88
2	2.61	4.28	4.11	0.61	0.96	0.63
3	2.94	3.72	3.71	0.77	1.00	0.77

Incidence of CL BC was significantly reduced for A vs T (p=0.007). The same improvements in DFS, TTR and CL BC have been observed in the subgroup of patients with ER+ and/or PR+ tumors. There were fewer distant recurrences reported for A (n=180) compared with T (n=203) and C (n=232), although the data for this endpoint are currently immature. The performance of tamoxifen in this trial reflected that seen in the EBCTCG world overview analyses (EBCTCG, *The Lancet* 1998; **351**: 1451-1467). In conclusion, A showed superior efficacy to T for DFS, TTR and CL BC. The benefits seen with A are attributed to improved drug activity rather than a sub-optimal effect of T. These results show A to be a highly effective treatment option for PM patients with early BC. For the first time there is a choice of adjuvant endocrine therapy for PM women with hormone responsive tumors. Longer follow-up will enable a more mature benefit/risk assessment to be made.

633 Beneficial side-effect profile of anastrozole compared with tamoxifen confirmed by additional 7 months of exposure data: a safety update from the 'Arimidex', Tamoxifen, Alone or in Combination (ATAC) trial

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ATAC, a randomized, double-blind trial, compared tamoxifen (T: 20 mg) with anastrozole (A: 1 mg) ('Arimidex') alone and the combination of anastrozole plus tamoxifen (C), as adjuvant endocrine treatment for postmenopausal patients with early operable invasive breast cancer. It included 9,366 patients who had completed primary therapy (surgery +/- chemotherapy). Radiotherapy could be given after randomization. Primary endpoints were disease-free survival and tolerability. The predefined adverse event (AE) data at the main analysis (MA) have been previously reported (The ATAC Trialists' Group. *Lancet* 2002; **359**: 2131–2139). An updated safety analysis (carried out in line with normal regulatory requirements) was performed after an additional 7 mths of follow-up. Table 1 reports the initial predefined AEs for which there were significant differences between treatments at the MA; additionally the 7-mth updated data for these AEs are reported. At the MA no significant differences in predefined AEs were seen between T or C. The efficacy data have not been updated.

Adverse events	**At main analysis**			**At updated analysis**		
	A (n [%]), N=3092	**T (n [%]), N=3094**	**Relative risk A/T**	**A (n [%]), N=3092**	**T (n [%]), N=3093**	**Relative risk A/T**
Median therapy duration (mths)	30.9	30.8		37.3	36.9	
Patients receiving 3-4 years of treatment (n)	937	927		1391	1322	
Patients receiving 4-5 years of treatment (n)	71	84		432	436	
Endometrial cancer	3 (0.1)	13 (0.5)	0.23	3 (0.1)	15 (0.7)	0.20
Vaginal bleeding	138 (4.5)	253 (8.2)	0.55	147 (4.8)	270 (8.7)	0.54
Vaginal discharge	86 (2.8)	354 (11.4)	0.24	94 (3.0)	378 (12.2)	0.25
Cerebrovascular events	31 (1.0)	65 (2.1)	0.48	34 (1.1)	70 (2.3)	0.49
Thromboembolic events	64 (2.1)	109 (3.5)	0.59	68 (2.2)	116 (3.8)	0.59
Hot flashes	1060 (34.3)	1229 (39.7)	0.86	1082 (35.0)	1246 (40.3)	0.87
Musculoskeletal disorders	860 (27.8)	660 (21.3)	1.30	936 (30.3)	732 (23.7)	1.28
Fractures	183 (5.9)	115 (3.7)	1.59	219 (7.1)	137 (4.4)	1.60

No major deviation was observed in the 7-mth updated AE profile from that of the MA for all predefined AEs. At the time of the MA, A was associated with significantly fewer withdrawals from treatment than T (21.9% vs 26.0%, P=0.0002), including significantly fewer withdrawals due to drug-related AEs (5.1% vs 7.2%, P<0.0001). The corresponding figures for the updated safety data were 24.1% vs 28.3% and 5.6% vs 8.1% respectively, demonstrating that the benefit in favour of A was maintained with longer follow-up. In conclusion, the 7 mths of additional data confirm the overall beneficial safety profile for A vs T and continue to provide reassurance of the safety of A following long-term treatment.

634 The effects of adjuvant anastrozole, exemestane, tamoxifen, and toremifene on serum lipids in postmenopausal women with breast cancer - a randomised study.

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Aim: The effects on serum lipids of tamoxifen (TAM) are well known. The data on toremifene (TOR) is more limited. Selective aromatase inhibitors anastrozole (ANA) and exemestane (EXE) are potential drugs for adjuvant treatment of breast cancer, yet their effects on serum lipids are poorly known. The aim of this study was to compare the effects of TAM, TOR (two doses), ANA and EXE on serum levels of lipids and lipoproteins, humoral growth factors, tumour markers, and blood coagulation factors in adjuvant treatment of breast cancer. The results from the lipid analyses are reported here.

Materials and Methods: Postmenopausal women with operable breast cancer were randomised into five adjuvant treatment groups, 30 patients in each: TAM 20 mg; TOR 40 mg; TOR 60 mg; ANA 1 mg and EXE 25 mg. A control group consisted of 30 postmenopausal women with a negative ER and PR status and to whom iv CMF chemotherapy was given as adjuvant treatment. Blood samples were drawn immediately prior to the treatment and after three months of therapy.

Results: There was no difference in the baseline mean values of serum cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides between the treatment groups. The drug effects on serum lipids of the three-month exposure to the hormonal agents and iv CMF chemotherapy are shown in table.

Drug	ΔTotal Chol %	ΔHDL-Chol %	ΔLDL-Chol %	ΔTrigly %
Anastrozole 1 mg	- 4.3	+ 1.6	- 7.8	- 0.3
Exemestane 25 mg	- 4.5	- 6.8	- 1.3	- 6.0
Tamoxifen 20 mg	- 14.8*	-5.1	- 22.6***	+ 8.1
Toremifene 40 mg	- 8.7	- 4.6	- 12.8	+ 3.8
Toremifene 60 mg	-12.0*	+ 7.3**	- 22.1***	- 15.9
iv CMF	+ 0.7	- 0.9	-0.5	- 7.4

*p<0.005 ; **p= 0.01; ***p=0.001 (One-Way ANOVA between groups).

Conclusions: The effects of TAM 20 mg and TOR 60 mg on the serum lipid profile are similar. The effects of ANA and EXE on the lipid profile in postmenopausal women are modest and may have little clinical relevance.

635 Adjuvant tamoxifen to premenopausal women. Results from a prospective randomized multicenter study.

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Background: Adjuvant treatment with tamoxifen reduces the risk of recurrence and breast cancer related death in patients with ER positive tumours irrespective of age and menopausal status. For premenopausal patients adjuvant endocrine therapy has not until recently been generally accepted. This is the first report from a randomized multicenter study of two years of adjuvant tamoxifen for premenopausal patients with stage II disease compared with no tamoxifen therapy.

Material and Methods: During 1984-1991, 565 premenopausal women with breast cancer stage II (pT1pN1, pT2pN1 and pT2pN0) were randomized to tamoxifen (n=276) or control (n=289). Steroid receptors were unknown in 19% of the tumours and 56% were ER and/or PgR positive and 25% were ER and PgR negative. In 506 (89%) of the tumours histological grading was re-evaluated by two of us (GC and ST).

Results: Histological grading was strongly correlated to prognosis for all patients as were the individual parameters in the histological grading. Tamoxifen therapy correlated with increased disease free survival for patients with receptor positive tumours of all histological grades. The tamoxifen effect on disease free survival (p=0.0089) was most marked for patients with receptor positive tumours and histological grade III (vs histological grade I-II). For receptor negative tumours we registered no effect of tamoxifen.

Discussion: Premenopausal patients with histological grade III receptors positive tumours (vs histological grade I-II) seems to benefit most from adjuvant tamoxifen treatment. This group has an increased risk of relapse and the positive effect of tamoxifen in this group is an important finding. It supports our previous data on the good effect of tamoxifen therapy on patients with high S-phase tumours (Breast Ca Res Treat 36; 23-34, 1995).

636 Toremifene and breast cancer therapy: incidence of secondary endometrial cancers.

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Background: Toremifene (Fareston) is a SERM antiestrogen drug globally indicated for the treatment of breast cancer. The drug has been in wide clinical use since 1995. Induction of secondary endometrial cancer has been the serious adverse event of major concern linked with the use of tamoxifen for breast cancer. Many separate studies have verified that tamoxifen increases the risk for endometrial cancer. The aim of the present study was to find out the incidence of endometrial cancers during toremifene trials and clinical use.

Study material: The accumulated safety data derived from toremifene clinical trials and the spontaneous adverse event report data file was carefully evaluated.

Results: Toremifene has been used for over 300 000 patient years. The cumulative incidence of reported endometrial cancers was low, about 0.07 cases per 1000 patient years. During toremifene trials (about 9000 patient years) the annual hazard rate was 1.0 per 1000 patient years. However, in 6/9 of the cases the toremifene therapy duration was less than 12 months before endometrial cancer detection.

Conclusions: This study suggests that toremifene therapy does not induce new endometrial cancers but rather reveals latent neoplasias shortly after the treatment onset. Based on the published data on clinical trials, the annual hazard rate for endometrial cancer has been about 2.0 for tamoxifen and 0.4 for placebo. Accordingly, endometrial cancer is not a safety problem of big concern during toremifene therapy of breast cancer.

637 Secondary leukemias after epirubicin-based adjuvant chemotherapy for operable breast cancer patients: 15 years experience of French Adjuvant Study Group.

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Background: Increased incidence of acute leukemia following chemotherapy (CT) with alkylating agents and/or topoisomerase II inhibitors has been reported. In patients (pts) treated with alkylating agents, FAB subtypes are generally M1-M2, and M4-M5 with topoisomerase II inhibitors.

Patients and methods: Between 1986 and 2001, 3633 assessable pts have been included in 8 FASG trials: 2589 received epirubicin (EPI)-based CT and 1044 received either hormonotherapy alone (n=682), either no systemic treatment (n=362). CT was as followed: FEC regimen in 2202 pts (5FU 500mg/m^2, EPI 50 or 75 or 100mg/m^2, cyclophosphamide 500 mg/m^2, 3 or 6 cycles every 21d), EPI-vinorelbine in 226 (EPI 50mg/m^2 d1, vinorelbine 25 mg/m^2 d1-d8, 6 cycles every 21d), EPI alone in 158 (EPI 30 mg d1-d8-d15, 6 cycles every 28d). EPI cumulative dose was <300mg/m^2 in 1603 pts, 300-600 in 823, and >600 in 79, followed by radiotherapy in 96% of cases.

Results: After a median follow-up of 8 years (max:15 yrs), 7 cases of leukemia occurred in EPI-exposed pts and 1 in non exposed pts. After 8 yrs, the risk to develop a leukemia was 0.3% (95%CI:0.17-0.43) in EPI-exposed pts, and 0.2% (0.03-0.37) in non exposed pts (p=.32) with a cumulative risk of 0.28% and 0.14%, respectively. In pts receiving CT, leukemia subtypes were: M2 (2), M3 (1), M4 (2) and acute lymphoblastic leukemia (ALL) (2). The imputability to CT was doubtful in 4 cases: 2 ALL and 1 M3 not classically related to CT, and 1 M2 occurring more than 10 years after CT. The only risk factor demonstrating a correlation was EPI cumulative dose: 6 cases (0.25%) in pts receiving <600mg/m^2, and 1 case of 79 pts (1.3%) receiving >600mg/m^2 (p=0.04).

Conclusion: The incidence of secondary leukemia after EPI-based CT was very low whatever the dose. Only 3 cases (0.1%) were probably related to CT. After a long follow-up, the benefit/risk ratio for operable BC pts was widely in favor of EPI-based adjuvant CT.

638 Leukemia incidence post treatment for primary breast cancer.

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Background: Leukemia incidence in breast cancer patients post radiation and/or chemotherapy treatment has been studied but the linkage to treatment regimens has been equivocal. The risk of leukemia post breast cancer treatment is not well defined.

Materials and Methods: A breast cancer registry of all patients seen at the community based institution was created in 1990 and is updated annually for recurrence and/or vital status, current as of the end of 2001. SEER registry data matched to the patient population was reviewed for completeness of leukemia case ascertainment. The analytic dataset consisted of females treated for primary breast cancer, stage 0, I, II, and III, with a minimum 24 months follow up (n=2949). Patients who did not have surgery, had incomplete chemotherapy data, non-standard chemotherapy treatment regimens, or a stemcell transplant were excluded (n=157).

Results: Average follow up time was 5.25 years (2 months to 12.34 years). Between 1992 and 1999 eight incident cases of all types of leukemia occurred (AML=3, ALL=1, CML=2, and CLL=2). Applying national age adjusted all type leukemia rates for women from 1996 to 1998 free of cancer at the beginning of the age interval to our 25 to 99 year old aged population (average age 63), 9 cases (.27%) would be expected. Using all cases of leukemia projected to occur in 2002 (SEER estimated rates) and applying those rates to our population we would expect 4.32 cases of AML/ALL. Cases of all type leukemia per treatment category were 1/156 (.6%) surgery/no chemotherapy (chemo)/no radiation therapy (xrt), 4/1457 (.3%) surgery/no chemo/xrt, 0/356 (0%) surgery/chemo/no xrt, and 2/977 (.2%) surgery/chemo/xrt. Restricting cases to Stage II and III, 3/1283 (.2%) all type leukemia cases were observed. There was 1/864 incident case in the adriamycin containing regimen treatment group and 1/420 in the CMF regimen group.

Discussion: There does not appear to be an excess risk of leukemia in the population based cohort treated at our institution. The observed rate of leukemia incidence was roughly equivalent to the expected rate across treatment categories including radiation and chemotherapy.

Supported by Kaplan Cancer Research Fund.

639 Late cardiac effects of adjuvant CMF vs CAF in women with node negative breast cancer treated on SWOG 8897: initial results from SWOG 9342.

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Background: Adjuvant therapy has increased the disease free and overall survival in early stage breast cancer. Little is known about the late cardiac effects of doxorubicin containing treatment regimens in this patient population. SWOG 8897 (INT-0102) was a phase III trial of adjuvant therapy in node negative breast cancer patients who received either 6 cycles of CMF or CAF (total doxorubicin dose 360 mg/m2), with or without tamoxifen. **Methods:** Disease free patients (Pts) initially enrolled from SWOG institutions were approached to participate in a study of late cardiac function (history, physical examination, radionuclide MUGA scan) between 5-8 years and 10 years after randomization to initial treatment. The primary endpoint was the number of women with a resting MUGA left ventricular ejection fraction (LVEF) < 50%. Secondary endpoints included other cardiac events and clinical symptoms which are obtained annually. **Results:** We report the results from the first assessment 5-8 years after randomization. 163 Pts were registered and 157 had MUGA scans (82 CAF; 75 CMF), representing 6% of pts registered to SWOG 8897. There was no difference between the two groups in menopausal status, breast cancer risk status or hormone receptors. Pts were similar to those treated in the parent study. 5% of CAF Pts had LVEF <50% and 7% of CMF Pts had LVEF <50% (P=NS); however, mean LVEF was 61.2% for CAF Pts and 64.9% for CMF Pts (P=0.006) There were no group differences in cardiac events or clinical symptoms. **Conclusions:** Pts recruited to SWOG 9342 had no evidence of serious cardiac dysfunction 5-8 years after adjuvant treatment with CAF in comparison to CMF. However, a significant difference in mean LVEF scores suggests poorer subclinical function in the CAF treated patients. We view this as an hypothesis generating finding. The 10 year MUGA scans and interval cardiac history will be used to evaluate this initial finding.

640 Cardiac toxicity in operable breast cancer patients after adjuvant chemotherapy with epirubicin: 7-year analysis in 3577 patients of French Adjuvant Study Group trials.

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Purpose: To compare the incidence of left ventricular dysfunction (LVD) before relapse, in operable breast cancer (BC) patients (pts) receiving (E+) or not (E-) epirubicin (EPI)-based adjuvant chemotherapy (CT).

Patients and methods: 3577 pts included in 8 FASG trials, between 1986 and 2001, were assessable for cardiac toxicity analysis: 2553 received EPI-based CT and 1024 received either hormonotherapy alone (n=662), either no systemic treatment (n=362). CT was as followed: FEC regimen in 2190 pts (5FU 500mg/m^2, EPI 50 or 75 or 100mg/m^2, cyclophosphamide 500 mg/m^2, 3 or 6 cycles every 21d), EPI-vinorelbine in 218 (EPI 50mg/m^2 d1, vinorelbine 25 mg/m^2 d1-d8, 6 cycles every 21d), EPI alone in 141 (EPI 30 mg d1-d8-d15, 6 cycles every 28d). EPI cumulative dose was <300mg/m^2 in 1478 pts, 300-600 in 910, and >600 in 79, followed by radiotherapy in 96% of cases. Median follow-up was 7.3 years.

Results: Between 1st CT cycle and 1 month after CT completion, 15/2553 pts (0.6%) developed LVD: in 13 pts, cardiac function was normalized or did not required further investigations, and in 2 pts, LVEF was stabilized. During follow-up period, 2 LVD occurred in E- pts (0.2%), and 18 in E+ pts (0.7%): in 14/18 pts, cardiac function was normalized or did not required further investigations, 1 pt was stabilized with specific treatment, 2 pts had cardiac worsening, and 1 died of congestive heart failure. The onset period was during the first 2 years with no case after the 9th year. After 7 years, the risk to develop a LVD was 1.36% (95%CI:1.10-1.62) in E+ pts, and 0.2% (0.06-0.36) in E- pts (p=.004). The study of risk factors demonstrated that only age was significantly correlated with LVD: 0.7% if <65 and 2.7% if >65 (p=.002).

Conclusion: After a 7-year follow-up (max:15years), EPI-related cardiac toxicity was very mild (1.36%) with only 1 toxic death (0.04%), and in 27/33 pts (82%), LVD was sporadic or well controlled.

641 Five year results of a randomised multicenter dose intense (DI-EC) study with Epirubicin (E) and Cyclophosphamide (C) in high risk breast cancer patients-a treatment of short duration with comparable efficacy to conventional chemotherapy.

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Efficacy of conventional adjuvant chemotherapy for high risk breast cancer patients with CMF or AC is limited. Triple drug or sequential regimens like CAF/CEF, AC-CMF, AC-T have significantly more side effects or longer treatment durations.

We randomised 183 breast cancer patients (pts.) (173 evaluable pts.) with ≥10 involved axillary lymph nodes (n=91) or extra capsular involvement (n=92) after primary surgery: arm A, standard combination 4 x E/C (90/600 mg/m^2) q3w followed by 3 x CMF (500/40/600 mg/m^2, d 1+8) q4w vs. arm B: dose dense regimen with 4 x (E/C) 120/600 mg/m^2 q2w with G-CSF support. All patients received post-operative radiotherapy. The median follow up is 63 months.

The applied weekly dose intensity of E was 28.7 mg/ m^2 in the standard arm and 54.7 mg/ m^2 in the dose-dense arm. DI-EC resulted in a significantly reduced treatment period: time on chemotherapy was 21.8 weeks vs. 8.9 weeks ($p<0.001$), total treatment time (surgery to end of therapy) was 6.5 months vs. 4.1 months ($p<0.001$).

Disease free survival (DFS) showed a clear trend in favour of DI-EC compared to EC/CMF (102 vs. 71 months (mo.) p= 0.14). Five year DFS-rate was 64 % vs. 50 %. 5-Year overall survival (OS) was 77 % vs. 65 % (p= 0.14). Time without symptoms or therapy (TWIST) was 98 vs. 64 mo. (p= 0.05). Toxicity and quality of life measured with standardised methods were similar in both arms.

Dose intensified chemotherapy with Epirubicin/ Cyclophosphamide with G-CSF reduced significantly the duration of therapy, while showing similar efficacy in DFS and OS compared to a conventional treatment regimen still recommended by international consensus meetings for adjuvant treatment of high risk breast cancer patients.

642 Phase III adjuvant trial of concurrent epirubicin/taxane vs. sequential epirubicin/cyclophosphamide followed by taxane for node positive breast cancer: safety analysis.

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Objective: To evaluate whether concurrent epirubicin and taxane (ET) improves disease-free survival (DFS) at 3 years compared to the sequential administration of epirubicin/cyclophosphamide followed by a taxane (EC followed by T) in patients with node positive breast cancer. Secondary endpoints: tolerability, safety, long-term DFS and overall survival. Enrollment goal: 600 patients anticipated in the fall of 2002. Interim safety analysis was performed on 310 patients enrolled between November 2000 and April 2002. Patients were randomized to concurrent ET (E 75 mg/m^2) for 8 cycles q21 days or sequential EC followed by T (E 90 mg/m^2, C 600 mg/m^2) for 4 cycles followed by 4 cycles of the chosen taxane q21 days. Physician discretion allowed for choice of taxane in either arm (T = paclitaxel 175 mg/m^2 or docetaxel 75 mg/m^2). Stratification was by age (<50 years, ≥50 years), nodal status (1-3, 4-10, >10 nodes), hormone receptor status and HER 2 status.

Results: 136 patients have been enrolled on ET (Arm A) and 144 on EC followed by T (Arm B). Patients are well balanced with respect to median age (52/53 years), tumor size and stage of disease. Two thirds of the patients (n=218) were treated with docetaxel and one third (n=92) with paclitaxel. To date, 107 patients have completed 8 cycles of therapy. Incidence of hematologic toxicities for the overall group: febrile neutropenia 12% vs. 3%; grade 4 neutropenia 58% vs. 56%; grade 3-4 thrombocytopenia 1% vs. 1% for Arms A and B, respectively. Grade 3-4 non-hematologic toxicities: nausea 3% vs. 5%; vomiting 3% vs. 8%; diarrhea 3% vs. 1% for Arm A vs. Arm B. Evaluation of LVEF was performed at baseline and the end of treatment. All patients had a LVEF of greater than 45% at baseline. Post-treatment, 1/42 and 1/49 patients had a LVEF below 45% without clinical symptoms.

Conclusion: This interim safety analysis indicates that both regimens are well tolerated. Patient follow-up is ongoing and further analysis of specific toxicity of each schedule and each taxane will be updated.

643 First follow-up data from interval-shortened dose-intensified adjuvant treatment with epirubicin/paclitaxel followed by CMF versus a standard treatment schedule in high-risk node-positive breast cancer patients (N+ 4 - 9 / > 9).

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Background: Dose density has been postulated as a major factor influencing outcome in the chemotherapy of breast cancer patients. We conducted a phase II/III trial to determine the safety profile of and to estimate the disease-free (DFS) and overall survival (OS) resulting from the combination of epirubicin/paclitaxel followed by CMF biweekly (with G-CSF support) vs. triweekly epirubicin/cyclophosphamide followed by CMF in high-risk breast cancer patients with 4 or more positive lymph nodes. Material and Methods: From June 1996 to November 2000, 225 primary breast cancer patients with 4-9 or more than 9 positive lymph nodes were recruited in this trial. Group A received 4 cycles of epirubicin 90 mg/m2 and paclitaxel 175 mg/m2 d1 every 2 weeks with G-CSF (5 µg/kg days 5-13) followed by 3 cycles of CMF (600/40/600 mg/m2 d1) biweekly. Group B was treated with 4 cycles of epirubicin/cyclophosphamide (90/600 mg/m2 d1) triweekly and 3 cycles of CMF (600/40/600 mg/m2 d1). Results: The regimen comprising epirubicin/paclitaxel followed by CMF was well tolerated as an intensified biweekly schedule with G-CSF support. Though side effects occurred more frequently in the intensified treatment arm, both regimens can be safely administered in high-risk breast cancer patients with more than 3 positive lymph nodes. The median follow-up data (30 months) showed for A (n=51) vs. B (n= 48) an absolute benefit of 9% for DFS and 6% for OS. The time to progression was similiar in both treatment groups. The actual analysis of all patients and subgroup evaluation will be performed at the conference. Conclusion: The dose-dense regimen with epirubicin and paclitaxel shows a trend towards higher DFS und OS.

644 Paclitaxel significantly improves the prognosis in ER-negative inflammatory breast cancer (IBC): the M.D. Anderson Cancer Center Experience (1974-2000).

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Background: The treatment of IBC now includes multidisciplinary management with anthracycline-based preoperative chemotherapy, surgery (S) and radiotherapy (XRT). In this study, we examine the role of adding paclitaxel to anthracycline-based regimens.

Materials and Methods: A total of 240 patients were treated in 6 consecutive clinical trials. Group A (n=178) was treated in the first 4 trials (1978-1993). With FAC (fluorouracil, doxorubicin, cyclophosphamide), FAC-CMF (cyclophosphamide, methotrexate, 5-flururacil), or FAC with vincristine and prednisone. Group B (n=62) was treated in 2 subsequent trials (1994-2000) with FAC followed by a q 3 week schedule of paclitaxel (FAC-P) or FAC plus high-dose weekly paclitaxel in the second trial (FAC-HDP). In all trials, the loco regional treatment consisted of radiotherapy and/or surgery. ER + or PR+ patients also received 5 years of tamoxifen.

Results: The two groups differed with respect to median f/u: A vs. B (%): 148 months (range 85-283) vs. 45 (range 21-99) and Estrogen Receptor (ER) status distribution, A vs. B (%), ER -: 58 (33) vs. 40 (65). There was no difference in the median age between the groups. The objective response rates (CR +PR) were similar (A=72%, B=79%). The 3-year overall survival (OS) and progression-free survival (PFS) were better in the patients treated with paclitaxel, but these differences did not reach statistical significance (OS: A, 53% vs. B, 71%, p=0.12), (PFS: A, 39% vs. B, 46%, P=0.19). However, for ER negative tumors the 3-year OS and PFS were significantly better when treated with paclitaxel (OS: A, 43% vs. B, 71%, P=0.035), (PFS: A, 31% vs. B, 39%, P=0.042).

Conclusions: Addition of paclitaxel to preoperative anthracycline-based therapy of IBC resulted in a trend for improved PFS and OS. This improvement reached statistical significance for ER negative patients. Paclitaxel should be included in the standard management of ER negative IBC.

645 Incidence of amenorrhea (A) in breast cancer (BC) patients (pts) ≤40 years of age after paclitaxel (T)-based adjuvant chemotherapy (CT).

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Background: Adjuvant CT prolongs disease-free and overall survival for BC pts, but also induces menopause (M). A quarter of all BC pts are pre-M and face CT-induced A with an increased risk for vasomotor, psychosocial, genitourinary, fertility, skeletal, and cardiovascular dysfunction. The impact of different CT regimens on subsequent ovarian function is uncertain. While there has been considerable documentation of the rates of CT-induced A with classical adjuvant regimens (see Table 1) there is little data for T in this setting.

Table 1: Incidence of CT-Induced A by Age Group

	<40 Years (%)	≥40 Years (%)	All Ages (%)
CMF-based **(1)**	40	76	68
FAC **(2)**	9	NA	NA
AC **(3)**	NA	NA	34

(1) Bines et al, J Clin Oncol 1996; 14(5):1718; **(2)**Sutton et al, Cancer 1990; 65:847; **(3)**Cobleigh et al, Proc Am Soc Clin Oncol 1995;14:A158;

Objective: To define the incidence of A in BC pts ≤40 years of age treated with adjuvant T-based CT with or without subsequent tamoxifen.

Methods: 196 pre-M BC pts ≤40 years of age treated with adjuvant T-based CT at Memorial Sloan-Kettering Cancer Center from 1997 to 2002 were identified. Eligible pts were required to have follow-up for at least 6 months after the completion of all CT. All study pts were treated with doxorubicin 60mg/m2 plus cyclophosphamide 600mg/m2 for 4 cycles and T 175 mg/m2 for 4 cycles, with or without tamoxifen afterwards. CT-related A was defined as a cessation of menses lasting ≥6months (Bines et al, J Clin Oncol 1996; 14(5): 1718).

Results: 102 pts met all eligibility criteria and had sufficient long-term follow-up. The median age of pts at the time of therapy was 36 years (range: 27-40yrs). Fourteen pts (14%) developed A and 88 (86%) resumed their menses. 53 pts (52%) also received tamoxifen: the incidence of A among them was 21% (11/53).

Conclusion: The addition of T to a standard adjuvant anthracycline-based CT does not appear to produce a high rate of CT-related A compared to historical controls. Prospective validation of this observation is warranted.

646 Sequential dose-dense epirubicin/paclitaxel (E-T) with G-CSF support compared to standard EC - T (epirubicin/ cyclophosphamide followed by paclitaxel) for patients with operable breast cancer and 1-3 positive lymph nodes- first toxicity analysis.

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Background: Dose-dense chemotherapy is predicted to be a superior treatment plan. Therefore we conduct a randomized trial to compare a dose-dense sequential treatment of epirubicin followed by paclitaxel with the conventional regimen epirubicin/cyclophosphamide followed by paclitaxel as adjuvant therapy in low-risk breast cancer patients (N+1-3).

Patients and Methods: Patients with resected breast cancer involving 1-3 axillary lymph nodes were treated in conventional 21d intervalls with EC-T (group A): 4x epirubicin (90mg/m^2, d1) /cyclophosphamide(600 mg/m^2, d1) followed by 4x paclitaxel (175 mg/m2, d1). Group B was treated with dose-dense E-T in 14 days intervalls: 4x of epirubicin (120mg/m^2, d1) followed by 4x paclitaxel (175 mg/m^2, 3h, d1) with obligatory G-CSF support (5 mg/kg G-CSF d5-10).

Results: Between May 2000 and May 2002 432 of 884 planned patients have been enrolled in this trial. To the date of abstract submission toxicity data are evaluable for 1537 treatment cycles (213 patients). Toxicities (CTC°) are as follows (% of cycles): EC-T vs E-T(+G-CSF): nausea/emesis (°3/4) <1% vs <1%; stomatits (°2/3) 3% vs 8%; PNP (°2) 4% vs 6%, PNP (°3) occured in < 1% of cycles in both arms; no grade 4 PNP was observed. Febrile neutropenia was observed in < 1% of cycles with EC-T and E-T(+G-CSF), respectively.

Conclusion: The first toxicity evaluation of this randomized study shows that the dose-dense sequential treatment of epirubicin (120mg/m^2) followed by paclitaxel (175 mg/m^2) q14d, with obligatory G-CSF-support and standard treatment with EC-T in 21 day intervalls (epirubicin 90mg/m^2 / cyclophosphamide 600mg/m^2 followed by paclitaxel 175mg/m^2) are comparably well tolerated. Longer observation of this trial will allow further assessment of tolerability and efficacy of the intensified vs conventional treatment regimens used here in the treatment of low-risk patients with resectable breast cancer and 1-3 positive lymph nodes. Updated results will be presented at the meeting.

647 Tolerability (toxicity, dose intensity and quality adjusted survival (QAS)) of ECMF versus CMF in the national epirubicin adjuvant trial (NEAT) of moderate risk early breast cancer.

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Background: The ***NEAT*** study compares Epirubicin (100mg/m^2 for 4 cycles) followed by classical CMF (4 cycles) with classical CMF (6 cycles) in women with moderate risk early breast cancer who require chemotherapy. All patients had CTC toxicity grades, dose intensity (mg/m^2/time) and supportive treatments recorded at each cycle. A representative subset of 509 women took part in a Quality of Life sub-study.

Results: 2027 patients were entered into the study. Prognostic characteristics were balanced across treatments: 69% were node positive; 61% <50 years old; 58% of tumours grade 3; 33% <2cms; 30% ER-ve; 47% ER+ve. Results show slight excesses of severe toxicities in the ECMF arm (p≤0.001) expressed respectively as ECMF%patients/CMF%patients: 15%/7% nausea, 12%/4% vomiting, 6%/3% stomatitis, 84%/27% alopecia and 7%/3% constipation. However, chemotherapy dose intensities generally exceeded 85%, indicating no major dose delays or reductions, and supportive treatments, myelosuppression, amenorrhoea, treatment-related mortality rates and global QAS were similar in both arms.

Conclusion: Despite recently available world-wide evidence supporting the role of anthracyclines in adjuvant therapy, there are still important groups of patients for whom there remains uncertainty. The importance of the tolerability and acceptance of the ***NEAT*** treatment regimens is a critical endpoint. Despite expected differences in acute toxicities, both regimens are shown to be tolerable (>85% delivered dose intensity) with similar global QAS.

648 Fast proliferating node negative breast cancer: combined analysis of two phase III trials comparing different adjuvant polychemotherapies vs control.

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Two national randomized phase III trials have recently analysed the clinical efficacy of polichemotherapy as adjuvant treatment for fast proliferating node negative breast cancer patients. IOR's study (J. Clin. Oncol., 2000; 18: 3125-3134) randomised patients to receive six cycles of CMF or no further treatment until progression after radical surgery; conversely, Bari's study (J. Clin. Oncol., 2001; 19: 3929-3937) randomised women to six cycles of adjuvant FEC or no further treatment. Patient entry criteria were similar in both studies (consecutive series of Tl-2NOMO; tumour proliferative activity analysed according to 3H- Thymidine Labe1ing Index, TLI; availability of ER and PgR status). The cut-off TLI values adopted to select women with fast proliferating breast cancer at high-risk for relapse were based on previous retrospectively validated analyses and performed in Laboratories partecipating the ad hoc Italian QC program.

Moving from 2056 new breast cancer patients treated during the accrua1 period (from 1989 to 1993), in the IOR study 281 patients were randomised; conversely, moving from 1336 new cases treated at NCI-Bari (from 1989 to 1994), 248 patients were randomised.

Results from each study and a stratified-by-study combined analysis of individual patients data from all randomised patients are shown in the table below.

Overall, the results from these two large controlled phase III trials confirm that: a) the tumour pro1iferative activity analysed according to 3H-Thymidine Labeling Index is usefull in selecting patients at high-risk for relapse candidate to adjuvant polichemotherapy; b) fast proliferating node negative breast cancer patients take benefit from adjuvant polichemotherapies either containing or not anthracyclines. The analysis of these benefits according to different drug regimens adopted will be presented.

	IOR Study		Bari Study		Combined Analysis	
	CMF	CTRL	FEC	CTRL	Treated	Ctrl
# pts	137	141	125	123	262	264
5-yrs DFS	83% *	72% *	81% **	69% **	82% ***	69% ***

* p=0.03; ** p=0.02; *** p=0.004

649 Concomitant or sequential chemo-radiotherapy (CRT) in operable breast cancer. Final results of a French multicentric phase III study.

Rouesse J, Cvitkovic F, De Lalande B, Serin D, Graic Y, Combe M, Leduc B, Lucas V, Demange L, Castera D, Krzisch C, Villet R, Garbay J-R, Nogues C. Centre Rene Huguenin, Saint-Cloud, France; Co-Participating Centers, France

Study design : concomitant adjuvant CRT (arm A : 4 FNC regimen mg/m2 5FU: 500 d1, Mitoxantrone: 12 d1, Cyclophosphamide: 500 d1, d21 with locoregional radiotherapy (RT) 45 to 50 Gy/5 weeks +/- boost 10 to 20 Gy) was compared in a phase III trial for node positive (≤ 7N+) breast cancer patients (pts) to sequential CRT (arm B : 4 FEC regimen mg/m2 5FU: 500 d1, Epirubicine: 60 d1, Cyclophosphamide: 500 d1, d21 before the same RT). Tamoxifen 20 mg/day x 5 yrs recommended for postmenopausal pts (≥ 50 yrs age). The study was powered to evidence a 10% difference in 5 yrs Disease Free survival (DFS) (α =0.05, 2 sided, β =0.20). Stratification done by center, nb of N+ (1-3/4-7) and type of initial surgery (tumorectomy (Tum)/ mastectomy (Mast)). **Pts and Results** : From 12/94 to 04/99, 650 pts accrued with 1.8% ineligible : median age 54 yrs, 60% menopausal status ; Tum : 65%, Mast : 35% ; N+ :1-3 : 81%, >3 : 19% ; no pronostic factors imbalance. Scheduled CRT completed in 94% (305/324) of pts in arm A vs 98% (309/ 314) in arm B (p=0.004), partly due to toxicities leading to discontinuation in arm A (9/19) vs in arm B (1/5). Significant acute toxicity in arm A vs arm B : febrile neutropenia (0.8% vs 0.08%, p=0.007), gr3/4 nausea-vomiting (4% vs 6%, p=0.04), gr2/3 alopecia (8% vs 49%, p < 10-7), gr2/3 skin local toxicity (29% vs 21%, p=0.02). No significant difference for the gr2 one year isotopic cardiotoxicity (LVEF) : 9/271 vs 4/265. Three yrs esthetic results differ only for local pigmentation 22% arm A vs 14% arm B (p=0.044). One case of secondary acute myeloid leukemia occured in each arm, at 3 yrs (arm A) and 1 yr (arm B). Five yrs efficacy, median follow up being 41 mo [2-72 mo] : DFS 83% in arm A vs 81% in arm B (NS) and overall survival 90% vs 87% (NS). No difference either in locoregional recurence or metastasis cumulated incidence. **Conclusion** : the concomitant CRT is feasable with tolerable acute toxicity. Except a shorter duration of the adjuvant treatment, this schedule doesn't offer any other advantage.

650 Does the timing of adjuvant chemotherapy after breast cancer surgery affect survival?

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Background: There are theoretical reasons to suspect that early initiation of adjuvant chemotherapy may improve survival for some patients. Retrospective analysis of IBCSG trials I, II, VI showed an improvement in 10 year disease-free survival (DFS) for premenopausal, node positive patients with estrogen receptor (ER) negative tumors who commenced chemotherapy within 3 weeks of surgery compared to those whose chemotherapy was commenced > 21 days following surgery (60% v 34%; p=0.0003) (Colleoni M, JCO 18:584, 2000). We have addressed the same question from our large single center database.

Methods: Between 1991 and 2001 1125 patients were treated with adjuvant chemotherapy for early breast cancer at the Royal Marsden. 686 patients received anthracycline-based chemotherapy and 439 received cyclophosphamide, methotrexate, 5-fluorouracil (CMF). The disease-free and overall survival of the 293 patients commencing chemotherapy within 20 days of surgery (A) was compared to that of the 832 patients commencing chemotherapy > 21 days after surgery (B). The median follow-up was 38 months.

Results: There were no significant differences in prognostic factors between the two groups apart from the number of patients with > 4 nodes involved (26% v 22%; p=0.04). There was no significant difference in 5-year disease-free survival between the two groups (69%A v 72%B; p=0.4). No significant difference in 5-year overall survival was found (81% v 84%; p=0.15). Early initiation of adjuvant chemotherapy did not improve disease-free survival for patients <50 years of age with ER-negative tumors (n=146)(58%A v 60%B; p=0.5). Cox multiple regression analysis was used to adjust the survival analysis for age, tumor size, number of involved nodes, grade, ER status, type of chemotherapy and use of adjuvant endocrine therapy. After adjusting for these factors the timing of commencement of adjuvant chemotherapy did not impact significantly on disease-free or overall survival for any patient sub-group analysed.

Discussion: We have been unable to identify a group of patients who derive a significant survival benefit from the initiation of adjuvant chemotherapy within 21 days of surgery including the subgroup of patients < 50 years with ER-negative tumors.

651 Effect of creatinine clearance (CrCl) on patterns of toxicity in breast cancer (BC) patients (pts) age 65 and older receiving adjuvant chemotherapy.

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Background: Although adjuvant chemotherapy for BC is widely used, age-specific patterns of tolerance and feasibility and baseline factors influencing risk of toxicity need to be further explored.

Methods: To determine toxicity from adjuvant BC chemotherapy as a function of age and CrCl we conducted a retrospective review of 1405 consecutive pts ≥ age 65 with primary invasive BC, seen in 1998-2000 at MSKCC. Inclusion criteria were: age ≥65, stage I, II, or III BC, and receipt of adjuvant chemotherapy at MSKCC [CMF (cyclophosphamide 600mg/m2 IV, methotrexate 40mg/m2 IV, 5-fluorouracil 600mg/m2 IV q3 wks x 8) or anthracycline based regimen: AC (doxorubicin 60mg/m2, cyclophosphamide 600mg/m2 q3 wks x 4), or ACT (majority received AC followed by Paclitaxel 175mg/m2 IV q 3 wks x 4)] with dose modification for cause. Exclusion criteria were history of prior chemotherapy, prior BC, or no baseline creatinine value at MSKCC. Data on 126 patients was available, mean age 69.7 (range 65-79) with stages: I (18%), IIA (40%), IIB (28%), IIIA (7%), IIIB (6%), T1Nx (1%). Pts were stratified by chemotherapy type: CMF (N=65) or AC/ACT (N=61). CrCl was calculated by Cockgroft and Gault (C&G) and Jeliffe.

Results: The majority of pts had a normal creatinine (mean 0.9; range 0.5-2.7; 96.8% with Cr ≤1.3). CrCl decreased with increasing age [increased age associated with decreased C&G (p=0.02); increased age associated with decreased Jeliffe (p=0.005)]. Despite this, decreased CrCl was not associated with increased risk of hospitalization, fever and neutropenia, dose delay, grade 3 or 4 heme or non-heme toxicity, treatment delay for low ANC, inability to complete chemotherapy course, or need for growth factors by univariate analysis. Increased age was associated with increased risk for fever and neutropenia, (p=0.006), dose delay (p=0.03), grade 3 or 4 heme toxicity (p=0.04), and need for GCSF (p=0.04) by univariate analysis.

Discussion: In this cohort of older breast cancer pts, CrCl decreased with increasing age; however, decreased CrCl as calculated by C&G and Jeliffe did not predict for increased risk of toxicity to adjuvant chemotherapy.

652 Adjuvant treatment of elderly breast cancer patients.

Neerukonda S, Young RR, Bingham AD, Keville LR, Riggs MW. Scott and White Memorial Hospital and Clinic, Temple, TX

Background: Breast cancer is the most common form of malignancy in women in the United States. SEER estimates 203,500 new cases of breast cancer in the year 2002 and 40,000 deaths. Approximately 30% of breast cancers occur in women over age 70. Previous studies in the late 1980' s and early 1990's have shown that older women are treated less aggressively and often were not offered standard treatment.

Materials and Methods: 520 women age 70 and above who were diagnosed and treated for their breast cancer at Scott & White from 1995-2000 were identified from the tumor registry data, then retrospectively reviewed. The data regarding the age, histological type, stage, treatment offered and received was collected by reviewing the charts, tumor registry data and the electronic medical records. 512 cases were analyzed and nine of these had a diagnosis prior to 1995 and eight had a diagnosis after 2000 leaving 495 women for analysis.

Results: Of the 495 patients, 63 patients (13%) were Stage 0, 245 patients (49%) were stage I, 122 patients (25%) were stage II, 29 patients (6%) were stage III, and 25 patients (5%) presented with stage IV disease. Chemotherapy was offered to 90 patients, 18% of the total. 71 of them accepted, and only 18 declined with 1 patient unknown. Hormonal therapy was offered to 301 patients (61%) and 267 women accepted, 32 declined and 2 unknown.

Discussion: Previous studies focusing on the treatment on the elderly breast cancer patients have shown they were treated less aggressively because of their age. Generally we expect that all patients with stage II through IV disease, and many stage I patients, should be offered systemic therapy. Our study concurs with the previous studies in that chemotherapy was offered to only 18% of the total patients, although about 35% of the total had stages of disease that would often be treated with chemotherapy. Hormonal therapy was offered to 61% of the patients, probably indicating a greater willingness to use therapy considered less toxic in the elderly women. Interestingly, of the patients that were offered chemotherapy, 79% accepted and only 20% declined. The percentage accepting hormonal therapy was even higher, with 89% of women accepting hormonal therapy and only 11% declining. These findings suggest that elderly women are not likely to decline therapy, but that women over age 70 are not being offered adjuvant systemic therapy as often as expected.

653 Outcome and prognostic factors in patients with early, hormone unresponsive, well differentiated breast cancer (pT1pN0G1-2) in the absence of adjuvant systemic treatment.

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Current guidelines (St. Gallen, NIH) recommend adjuvant endocrine therapy for all hormone responsive (HR+) tumors. However, it remains unclear, which pts. with small, HR(-) tumors benefit from cytostatic treatment. The purpose of this study was to evaluate the outcome of pts. with HR(-), pT1, pN0, pM0, G1-2 tumors in the absence of adjuvant systemic treatment.

Since 1982, 200 pts. with negative estragon and progesterone receptors were treated for breast carcinomas ≥ 2 cm. All tumors were well or moderately differentiated (G1-2). Systematic axillary dissection ruled out axillary lymph node metastases in all pts. None of the pts. received systemic treatment. Pts. with unknown HR status or grading were excluded. Data were contemporaneously collected and pts. were followed for a mean of 68 months. The median age was 58 years, 14 pts. (7%) had pT1a tumors, 43 (22%) pT1b tumors and 143 (71%) pT1c tumors. Histopathological grading was excellent in 41 pts. (G1, 21%) and moderate in 159 pts. (G2, 79%). Peritumoral lymphangiosis carcinomatosa (PLC) was present in 22 pts. (11%). Among 24 deaths (12%), 13 (7%) were associated with breast cancer, while 11 deaths (6%) were of other causes. The overall survival (OS), as estimated by Kaplan-Meier analysis, was 206 mon (95% CI, 173-240), if censored for cancer associated death causes, and 217 mon (192-242) for non-cancer associated death causes. In multivariate analysis, allowing for tumor size, age, grading and the presence of PLC, only PLC was an independent prognostic parameter (RR 6.17 [1.95-19.50], p=.005). Censored for cancer associated death, the mean OS in elderly pts. (≥ 60 years) without PLC was significantly greater (185 mon [126-174], 5 y OS 92.7%), than the OS censored for other death causes (150 mon [174-197]).

In the absence of PLC, pts. ≥ 60 years of age with HR(-), early breast cancer (pT1pN0G1-2) are more likely to die of other causes than of their malignant disease. Considering decreased efficacy of cytostatic treatment and the given prevalence of co-morbidity in this age group, these pts. most likely will not profit from adjuvant chemotherapy.

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ABSTRACT #654

WITHDRAWN

655 Characteristics of Korean breast cancer in women undergoing hormone replacement therapy.

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Background: Hormone replacement therapy(HRT) has been associated with an increased risk for breast cancer. Cancers in women who undergoing HRT are often less advanced, and a lower mortality has been reported in those who use HRT vice in nonusers. We sought to explain this by a comparison of indicators of tumor aggressiveness in patients who received HRT with those in patients who did not.

Materials and Methods: A population-based cohort of 370 postmenopausal women with breast cancer were interviewed for the use, type, and duration of HRT. Clinical variables and indicators of tumor aggressiveness(nuclear grade, hormone receptors, c-erb-B2 overexpression, tumor size, lymph node) were analyzed.

Results: Breast tumors from 268 HRT patients were smaller(p=0.001), had less involved axillary lymph nodes(p=0.0),and had a lower overexpression of c-erb-B2(p=0.047) than the tumors from 102 non-recipients. These differences persisted after adjustments for age at diagnosis and screening with mammography by multiple logistic regression. No significant differences were observed in estrogen(ER) or progesterone receptor content(PR), or nuclear grade. Neither the type of HRT(estrogen versus combination of estrogen and progesterone), nor the duration of HRT was associated with the tumor size or with the involvement of lymph nodes. The use of HRT was significantly associated with a longer metastasis free survival in women with breast cancer,(p=0.028) but was not associated with longer overall survival. The use of HRT was not significantly associated with longer overall survival or with a longer metastasis free survival after adjustment for T-stage, N-stage, age at diagnosis or screening mammography.

Discussion: The results indicate that breast cancer in women who receive HRT is biologically less aggressive than those without previous HRT. This may at least partly explain why breast cancer in HRT users has a more favorable clinical course.

656 Prevention of bone loss in the ovariectomized rat model by combined treatment with SCH57068, Premarin, and DHEA.

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An ideal approach at menopause is to prevent breast cancer, uterine cancer, cardiovascular disease and osteoporosis. Postmenopausal osteoporosis can be reduced by hormone replacement therapy but some undesirable effects are associated with chronic estrogen and progestin administration. We have studied the preventive effect on bone loss of a combined treatment with the SERM SCH57068, an estrogen (Premarin) and dehydroepiandrosterone (DHEA) in ovariectomized female rats. Ten- to 12-week old female Sprague-Dawley rats were ovariectomized (OVX) and treated for 7 months with SCH57068 (2.5 mg/kg; oral), Premarin (0.5 mg/kg; oral) and DHEA (80 mg/kg, topical application). Bone mineral density (BMD) of lumbar spine (LS; vertebrae L2-L4) and femur (F) was measured after 7 months of treatment, using dual energy X-ray absorptiometry (DEXA; QDR 4500A, Hologic). LS BMD was 17% lower in OVX control rats than in the intact group ($p<0.01$). The administration of SCH57068, Premarin or DHEA alone prevented LS BMD decrease by 68%, 64% and 51% (all $p<0.05$), respectively, while the combined treatment with SCH57068, Premarin plus DHEA completely prevented LS BMD loss caused by the ovariectomy (LS BMD of 0.2492 g/cm2 versus 0.2494 g/cm2 in intact control animals). Similar results were observed on F BMD. Moreover, total body fat percentage was increased from 29% in intact control animals to 40% in OVX rats while the administration of the combined treatment with SCH57068, Premarin and DHEA led to a fat percentage value of 29%, thus completely preventing the OVX-induced increase in fat percentage. Serum cholesterol levels were also decreased by 70% (compared to OVX) following the simultaneous administration of the three compounds. Considering the pure and potent antiestrogenic activity of SCH57068 in the mammary gland and endometrium, and the potential beneficial effects of estrogens (Premarin) and androgens (DHEA) on vasomotor symptoms, well-being and lipids, these preclinical data suggest that such a combined therapy could be advantageously used to prevent osteoporosis while providing beneficial effects for the prevention and/or treatment of breast and uterine cancer and improving the lipid profile. The addition of an androgenic compound (DHEA) could offer a more physiological hormonal replacement therapy while further protecting against breast cancer.

657 No difference in menopausal symptoms and severity of hot flashes (HF's) comparing combined hormone replacement therapy (HRT) plus tamoxifen (Tam) to tamoxifen alone in peri- or postmenopausal women at high risk for developing breast cancer.

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It is not known whether women taking combined Tam/HRT have fewer menopausal symptoms than those taking Tam alone. 181 peri- or post-menopausal women at high risk for developing breast cancer (5-year Gail score > 1.7%; breast biopsy of LCIS, DCIS or atypical hyperplasia) were treated with Tam, 20 mg QD, alone (N= 95), mean age 53; or remained on HRT and began treatment with Tam (N=86), mean age 54. Pts' menopausal symptoms were measured at baseline (on nothing or HRT) prior to beginning Tam, at 3 mos. after beginning Tam, and again at 1 year. Menopausal symptoms (vaginal dryness, mood alteration, insomnia, low energy, impaired verbal fluency, diminished libido, etc) were rated by the pts as none (0), mild (1), moderate (2), or severe (3) and the HF's were quantitated using the product of a categorized number of HF's/day and intensity.

The median scores at baseline, after 3 mos., and after 12 mos. on Tam vs Tam/HRT are summarized in the table below. In 59 pts (38 Tam alone, 21 Tam/HRT) on therapy for 1 year to date, 24% vs 24% of women on Tam vs Tam/HRT had gained 1 to 5 lbs; 21% vs 0% had gained 6-10 lbs; 0% gained 15-20 lbs; and 11% vs 5% gained 20+ lbs. 1 pt developed superficial thrombophlebitis on Tam, and 1 pt each had a TIA or MI on Tam/HRT. 16 pts on Tam alone discontinued therapy vs 10 who stopped Tam on Tam/HRT.

Conclusion: Combining Tam and HRT does not prevent the HF's associated with Tam in postmenopausal women. Significant weight gain may be less with combined Tam/HRT.

Median Menopausal Symptom & Hot Flash Comparison

	BASELINE		3 MONTH		1 YEAR	
Study Arm	Tam	Tam/HRT	Tam	Tam/HRT	Tam	Tam/HRT
Menopausal Symptoms	4.4 n=95	5.7 n=84	4.0 n=76	5.8 n=55	5.3 n=38	5.7 n=21
Hot Flash Scores (categorized #/day x intensity) Categories: 0, 1(1-4), 2(5-9), 3(10-14), 4(15+) Intensity rated 1 to 4	1.7 n=95	0.7 n=83	2.4 n=77	2.4 n=55	2.7 n=38	2.3 n=21

Supported by a research grant from AstraZeneca

658 CerbB-2 oncoprotein expression does not affect biological response to oestrogen deprivation in ER positive cancers.

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Breast cancers expressing cerbB-2 oncoprotein respond poorly to tamoxifen and other endocrine therapy.

Aim: To determine if oestrogen deprivation (by withdrawing HRT) reduces epithelial proliferation in cerbB-2 positive and negative tumours.

Methods: Women who developed breast cancer whilst on HRT opted to either continue HRT until surgery (n=15) or stop HRT (n=125) at the time of diagnosis by core biopsy. Ki67 (a measure of cell proliferation), ER (Oestrogen receptor), PR (Progesterone Receptor) and CyclinD1 were measured by immunohistochemistry on paired sections of core biopsy and operative specimens for each patient. cerbB-2 immunostaining was scored from 0-3 (Novocastra NCL-CB11).

Results: Overall 106 ER positive and 19 ER negative women stopped HRT. Stopping HRT led to a significant fall in epithelial proliferation only in ER positive patients. ($p<0.001$: Paired t-Test ; see table).Women who continued HRT had a rise in cell proliferation (P=0.01) from mean core13.47 (range2.92 to 34.5) to operation15.64 (range2.28 to 41.78). Fall in PR ($P<0.001$) and Cyclin D1 expression ($P<0.001$) were seen in responding tumours following withdrawal of HRT regardless of cerbB-2 status

Conclusion: Withdrawal of HRT leads to decreased cell proliferation and PR expression in ER positive tumours regardless of cerbB-2 expression. HRT should be stopped at diagnosis of breast cancer.

CerbB-2 status (No:106)	Median Ki67 change (range)	Median PR change(range)	Median CyclinD1 change(range)
Negative(0) 60	-6.1 (-16.1to7.9)	-6.5(21.2to12.8)	0(-32.9to4.8)
Negative(1) 31	-6.2 (-14.1to12)	-11.5(-22.2to2.6)	-6.25(-28.4to3.2)
Positive(2) 12	-4.4 (-12.9to1.8)	-8.7(-16.4to2.5)	-1.1(-10.1to 12.8)
Positive (3) 3	-21.3 (-38.8to-18.3)	-6.8(-6.8to-6.8)	No Expression
kruskall-wallis Test	P=0.80	P=0.26	P=0.35

659 The characteristics of malignant breast tumors in HRT users compared to those in non-users.

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Background: Hormone replacement therapy (HRT) has gained wide popularity among women in menopause. The relationship between HRT and breast cancer is controversial. A large amount of data suggests that HRT in menopausal women is a risk factor for breast cancer. However, the nature of malignant tumors, which are diagnosed during HRT therapy is not clear.

Materials and Methods: Between May 2000 and March 2002, 302 patients were diagnosed with breast cancer at The Comprehensive Breast Care Center at Assaf Harofeh Medical Center. All were interviewed and completed a personal questionnaire regarding medical and hormonal history, including the use of HRT. Every woman who used systemic HRT for more than one year, and up to 5 years prior to the diagnosis of breast cancer was considered to be an HRT user.

All patients aged 50-75 years were evaluated, and we compared the characteristics of the tumors diagnosed in HRT users and non-users.

Results: 302 women were diagnosed as having breast cancer during the above mentioned period., 167 of whom were between the ages of 50-75 years and were available for evaluation. 36 out of these 167 patients used HRT (21.4%). All used combined therapy with estrogen and progestins. The disease stages (HRT-users vs. non-users) are shown in Table 1.

The disease pathological grading (defined as a percentage of the whole group) was: HRT-users vs. non-users: grade I - 32%, 9%; grade II - 52%, 56.2%; grade III - 16%, 34.8% respectively. In invasive tumors the rate of estrogen receptor positive in users vs. non - users was 75%, 89% and Her-2neu positive (+2/+3), 16%, 18.5% respectively.

Conclusions:

1.The rate of HRT users in our group is similar to that found in Israel.
2. No advanced stage disease was found in HRT users (stages III and IV).
3. The grade of tumors was lower in HRT users.
4. Rates of ER positive and HER-2-neu positive tumors were similar in both groups.

Table 1: Disease Stages (HRT-users vs. non-users)

	DCIS	I	II	III	IV
+HRT	5/36 (14.3%)	14/36 (38.9%)	17/36 (47.2%)	0/36	0/36
-HRT	18/132 (13.6%)	54/132 (40.9%)	44/132 (33.3%)	13/132 (9.8%)	3/132(2.3%)

660 Effects of exercise training on plasma insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) in postmenopausal breast cancer survivors: results from the REHAB randomized controlled trial.

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Background: Epidemiologic data indicate that high levels of IGF-1 and low levels of IGFBP-3 may be associated with an increased risk of breast cancer and adverse prognostic factors. Interventions that modify IGF-1 and IGFBP-3 may modulate breast cancer risk, and disease recurrence and progression in breast cancer survivors. The purpose of the current study was to determine the effects of exercise training on changes in plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio concentrations in postmenopausal breast cancer survivors.

Methods: 53 postmenopausal breast cancer survivors were randomized to either an exercise group (n=25) or control group (n=28). The exercise group trained on cycle ergometers 3 times per week for 15 weeks. The control group did not train. Plasma concentrations of IGF-1 and IGFBP-3 were measured at baseline and postintervention with enzyme-linked immunosorbent assay kits (Quantikine, R&D Systems, Inc., Minneapolis, Minn). The interassay variations were 5.1% and 3.0%, respectively. The IGF-1/IGFBP-3 molar ratio was calculated as IGF-1 divided by IGFBP-3. Independent-samples t-tests were used to compare changes between groups from baseline to postintervention.

Results: 52 participants completed the trial. The exercise group attended 98.4% of the prescribed exercise sessions. Baseline values for plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio concentrations did not differ between groups. IGF-1 decreased by 4.9 ng/ml in the exercise group whereas it increased by 2.5 ng/ml in the control group (mean difference, -7.4 ng/ml; 95% CI, -14.6 to -0.2 ng/ml; p=.045). IGFBP-3 increased by 103.4 ng/ml in the exercise group whereas it decreased by 77.1 ng/ml in the control group (mean difference, +180.5 ng/ml; 95% CI, 28.4 to 332.5 ng/ml; p=.021). The IGF-1/IGFBP-3 molar ratio decreased by 0.003 in the exercise group whereas it increased by 0.003 in the control group (mean difference, -0.006; 95% CI, -0.01 to -0.001; p=.017).

Discussion: Exercise training had a beneficial effect on plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 molar ratio concentrations in postmenopausal breast cancer survivors. Further randomized trials are planned to determine whether these effects are associated with clinical endpoints.

661 Frequency of diagnostic and therapeutic interventions during the terminal phase of metastatic breast cancer-differences in medical management between academic institutions and a hospice.

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Most patients, who face the terminal phase of their malignant disease, prefer a familiar surrounding with professional, but contained medical support. Purpose of this study was to evaluate differences in the preference for diagnostic and therapeutic interventions between an academic hospital institution and a hospice institution during this stage of disease.

At two academic hospital institutions (I. Frauenklinik Munich and Klinik Oberaudorf, n=326) and at a hospice (Krankenhaus der Barmherzigen Brüder Munich, n=99), the hospital charts of patients with breast, uterine and ovarian malignancies were reviewed for diagnostic and therapeutic interventions during the four final weeks. In addition, we evaluated the control of symptoms and the involvement of spouses in the final phase before the patient deceased.

At the academic hospitals, radiological tests, such as x-ray were performed in 301 patients (92.3 %), while in 127 patients (39.0 %) intensified diagnostics, such as computerized tomography were conducted. In comparison, at the hospice institution, no intensified radiological tests were performed at all, and standard radiographies were undertaken in only 8 patients (8.1 %, $p < .0001$, c2-test). During the final four weeks of life, multi-agent chemotherapies were applied to 145 patients (44.5 %) at the academic hospital, while no patient received chemotherapy at the hospice ($p < .0001$). Major bothering symptoms could not be treated successfully in 64 patients dying (19.6 %) at the academic hospital and in 7 patients (7.1 %) at the hospice ($p = .003$). During the immediate phase before the patient passed away, spouses were present equally in the examined institutions, both at the academic hospital ($n = 115$, 35.3%) and at the hospice ($n = 37$, 37.4%, $p = .70$) The high incidence of interventional measures during the final phase of life at academic hospital institution may reflect patient's inadequate expectations for cure or significant prolongation of their life, caused by a lack of sufficient counselling. In contrast, an informed consent with the educated patient might lead to a more successful optimisation of the quality of life during the terminal stage of malignancies.

662 Ovarian stimulation and in vitro fertilization with tamoxifen for the prevention and treatment of chemotherapy-related infertility in breast cancer patients.

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Background: Breast cancer is the most common malignancy in reproductive age women. Because of the probability of ovarian failure and infertility as a result of chemotherapy, increasingly more women are considering fertility preservation. Because increased estrogen levels are thought to be potentially risky in breast cancer patients, embryo cryopreservation after unstimulated cycles (NCIVF) has been traditionally considered as a safe fertility preservation strategy. NCIVF, however, typically results in the generation of a single embryo.

Methods: In this study, we employed tamoxifen as an ovulation induction agent to perform in vitro fertilization (TamIVF) and compared it to a control group of cancer patients ($n = 5$) who had in vitro fertilization without ovarian stimulation (NCIVF, 9 cycles). Twelve women with ductal carcinoma in situ or invasive breast cancer stage 1-3 received tamoxifen at 40-60 mg for 5-12 days (6.9 ± 0.6) beginning on the 2nd-3rd day of their menstrual cycle (15 cycles). In vitro fertilization via intracytoplasmic sperm injection was performed as per standard clinical protocols.

Results: Cycle cancellation was significantly infrequent in TamIVF, compared to NCIVF (1/15 vs. 4/9, $p < 0.05$). Compared to NCIVF, TamIVF patients had a significantly greater number of mature oocytes (1.6 ± 0.3 vs. 0.7 ± 0.2, $p = 0.03$) and embryos (1.6 ± 0.3 vs. 0.6 ± 0.2, $p = 0.02$) per initiated cycle. Tamoxifen stimulation resulted in the generation of embryo(s) in every patient (12/12) while only 3 out of 5 patients had an embryo following NCIVF. Of the 12 patients in the TamIVF group, 8 intended embryo cryopreservation in 9 cycles, prior to chemotherapy; while 4 patients attempted pregnancy with fresh embryos in six cycles, post-chemotherapy. Of the six fresh transfer cycles in TamIVF, 2 resulted in pregnancy, and one patient delivered a healthy infant. After a mean follow up of 16 ± 3.6 months (4-55 months), none of the patients had cancer recurrence.

Discussion: Tamoxifen-IVF may provide a safe method of ovarian stimulation and fertility preservation in breast cancer patients. We reported the first live birth with fresh embryo transfer after tamoxifen stimulation; longer follow up will be required to determine the pregnancy rates with cryopreserved embryos after TamIVF.

663 A survey of women with breast or gynecological cancer: Parcours de Femmes 2001.

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A survey of women with breast cancer or gynecological malignancies was conducted in France in 2001. The purpose of the study was to examine patients' (pts) perceptions of the cancer experience, and the degree to which their needs and expectations regarding health care were met. A questionnaire was completed by pts with newly diagnosed or relapsed disease who were 3 months minimum into treatment or were in follow up phase (completion or treatment < 1 year). A parallel survey was undertaken on health care personnel (oncologists, nurses, radiotherapy technicians) in order to compare their perceptions on health care delivery with those of the pts. Among 1870 pts (corresponding to a response rate of 66%), 87% and 9% had breast and ovarian cancer, respectively, 76% of pts were de novo, 24% in relapse, 92% of pts received an information about their cancer at the time of diagnosis: 19% and 34% of pts with newly diagnosed and relapsed disease respectively found this information inadequate. 81% of pts could ask all their questions to the medical staff, however only 33% of relapsed pts could obtain all information on their treatment. With regard to their therapy, 95% of pts were satisfied with the given information on the type of treatment, 77% about the side effects of the treatment. 79% of pts who were given the option of participating in a clinical trial accepted the proposition. 31% of the pts had consulted at least a second physician prior to start of treatment. Among pts having a professional activity at the time of diagnosis, 66% had stopped working. The problems most frequently cited were fatigue 78%, nausea and vomiting 61%, alopecia 63%, anxiety 66%, sleep disorders 48%. The participation rate of 66% in this survey was higher compared to previous surveys in France (Parcours de Femmes in 1993, 28%) and Europe (CAWAC survey in 1997-98, 43%) suggesting an increase of communication skills in women with cancer regarding their disease and surrounding issues. This study also highlighted the need to improve the quality of information given to relapsed pts and the need for a program to manage all non-medical means in and outside comprehensive cancer centers.

664 Survival benefit of appropriate treatment options in elderly women > 80 years with breast cancer.

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Background: Breast cancer is a major health issue and the incidence increase with age. Treatment is controversial in the elderly, particularly over 80. Our objective was to evaluate treatments and their effect on survival. Material: Between 1980 and 1999, 5392 breast cancer patients (pts) were treated at regional facilities and followed by a cancer registry. We identified 407 women over 80. Clinical and pathological features, health status, co-morbid conditions, treatments and survival were evaluated. Mortality from breast cancer and other causes were considered separately. Determinants of treatment were studied by logistic regression. Comparisons of survival were analyzed by Cox models adjusted for others prognostic factors. Results: More than 47% of pts were over 85. The stage distribution was as follows: I in 91(22%), II in 169(42%), III in 61(15%) and IV in 37(9%). Co-morbid conditions were absent in 8%, acute in 39%, chronic in 34%, unknown in 20%. Twelve percent (n=48) did not received treatment, 32% (n=132) had tamoxifen only, 7% breast conserving surgery(BCS) alone (n=28), 14% mastectomy (n=55), 14% (n=57) BCS plus adjuvant therapy, 19% (n=78) mastectomy plus adjuvant therapy and atypical treatments in 2%. At last follow-up, 235 deaths were recorded, 98 due to breast cancer and 137 to other causes. Five- years overall observed survival was 34%(95%CI: 29-39%) and 5-years specific survival was 65%(95%CI: 59-71%). Breast cancer mortality decreased progressively between the following therapeutic groups: no treatment, tamoxifen alone (adjusted HR: 0.4, 95%CI: 0.2-0.7), BCS alone (HR: 0.4, 95%CI: 0.1-1.4), mastectomy alone (HR: 0.2, 95%CI: 0.1-0.7) or with adjuvant treatment (HR: 0.2, 95%CI: 0.1-0.5), BCS plus radiotherapy or other adjuvant treatment (HR: 0.1, 95%CI: 0.03-0.4). Atypical treatment were associated with a poor prognosis, similar to therapeutic abstention (HR: 0.8, 95%CI: 0.2-2.5). Conclusion: Treatment options strongly modify the prognosis of breast cancer among elderly women. Treatments need to be adapted to patients general status and co-morbid conditions, but have to offer the best chance of cure, regardless of patients age.

665 Retrospective study of the effect of comorbidity on use of adjuvant chemotherapy in older women with breast cancer in a tertiary care setting.

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Background: Use of adjuvant chemotherapy for breast cancer decreases with increasing age. In a retrospective analysis, we examined the effect of comorbidity on adjuvant chemotherapy use in postmenopausal women.

Materials and Methods: We retrospectively reviewed clinic charts of new breast cancer patients over age 55 seen between 1990 and 1995. Information gathered included: demographics; tumor characteristics; treatment delivered; and comorbidity (number and presence of heart, respiratory, liver and gallbladder disease, other cancer, and diabetes). Multivariate analysis was used to determine the effect of age, tumor stage, and comorbidity on use of adjuvant chemotherapy.

Results: There were 273 charts reviewed: 127 aged 55-64 years (mean age 57.2 years) and 146 aged 65 years and older (mean age 72.4years). Older women were more likely to be single (50% vs 13.9%, p<0.001), live alone (23.5% vs 3.2%, p<0.001), have a greater mean number of comorbid illnesses (1.48, range 0-6 vs 0.85, range 0-5; p<0.001), and have respiratory disease (3.1% vs 10.3%, p=0.02). There was a trend toward more cancers in the older group (11.0% vs 4.7%, p=0.06). The groups were similar with respect to other comorbidities, tumor stage (p=0.13), number of involved lymph nodes (p=0.74), and estrogen and progesterone receptor status (p=0.07 and 0.48, respectively). Older women were more likely to have mastectomy (86.1% vs 76.2%, p=0.04). Tamoxifen use was similar in both groups (82.8% vs 81.1%, p=0.72). Chemotherapy use was less frequent in older women, regardless of nodal status: overall, 13.1% vs 45.6%, p<0.001; node-(-), 8.2% vs 32.1%, p<0.001; and node-(+), 25% vs 83.8%, p<0.001. In a multivariate analysis, stage (p<0.001) and age (p<0.001), but not comorbidity level (p=0.11), predicted use of adjuvant chemotherapy.

Discussion: In this group of postmenopausal women, level of comorbidities did not explain less frequent use of adjuvant chemotherapy. Although this study is limited by the retrospective design, number of patients, and generally low level of comorbidity, it is consistent with other studies finding that age itself predicts use of adjuvant chemotherapy in older women.

666 High-throughput quantitative proteomics of human breast cancer tissue using 2D-differential in-gel electrophoresis and mass spectrometry.

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Background: The determination of true changes in the progression of human breast cancer at the molecular level remains a significant challenge. Characterizing disease-related expression changes in pure cell populations and correlating them with those observed in human tissue samples will help our understanding of the changes in protein expression important in disease prognosis. Using laser capture microdissection, two dimensional differential in-gel electrophoresis (2D-DIGE) and bioinformatics tools, we have compared protein expression profiles in protein homogenates from biopsy samples to detect disease related expression changes, and separate them from changes due to natural cellular heterogeneity.

Materials and Methods: Laser microdissection (LMD) was performed on a Leica AS LMD instrument. Briefly, fresh frozen, OCT-embedded 5-10 micron tissue sections were cut and stained with Toluidine Blue to allow cellular visualization. Proteins from frozen biopsy samples were isolated following homogenization in buffer (urea / thiourea, pH 8.5), labeled with Cy dyes (Cy3, Cy5) and focused on IPG strips (pH 4-7) and separated on 12% polyacrylamide gels. Gels were scanned for Cy3/Cy5 using a Typhoon 9400 imager. Spot excision, protein digestion and MALDI target spotting was performed on an automated spot handling workstation. Peptide mass fingerprinting was carried out by MALDI MS, and/or ES MS/MS.

Results and Discussion: Comparisons of normal breast tissue against tissue diagnosed as fibroadenoma, IDCA, DCIS revealed differences in protein expression over a wide dynamic range. Control comparisons between different tissue samples of the same classification also revealed differences which we have differentiated by principal component analysis. The obtained 2D profiles were also compared to 2D-DIGE analyses of enriched tumor cell populations, procured by laser microdissection of freshly frozen OCT-embedded biopsy sections. Decyder analysis showed that up to 2.3% of LMD-procured proteins exhibited threefold or greater differences in protein expression. In one run, 576 spots were marked for picking from 5 gels. Spot picking, digestion and MALDI target spotting were completed in 23h. 385 proteins (66%) were successfully identified using the Ettan Pro MALDI. Of the remainder, 15% were identified by ES-MS/MS.

667 Galectin-3 accumulates in aggressively growing breast stromal tumors.

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Galectin-3, a b-galactoside binding lectin, accumulates upon malignant transformation of mesenchymal-derived cells and is associated with experimental sarcoma tumor progression. In this study, we have evaluated the accumulation of galectin-3 in two types of breast stromal tumors, namely fibroadenomas (FA, n = 71) and Phyllodes tumors (PT, n = 65), using immunohistochemistry. Microenvironmental parameters associated with angiogenesis, such as VEGF and PDGF, NO synthase production and accumulation of p53 in stromal cells were also evaluated. Among FA, 28/71 were juvenile fibroadenomas. PT was classified according to WHO, taking stromal cell mitotic index into account: 42/65 were low-grade, 18/65 were intermediate and 5/65 were high grade lesions. Stromal cells tended to accumulate galectin-3 mainly in the nucleus. About 43 % of the juvenile FA accumulated galectin-3, while only 9.3% of the FA lesions of adult women were positive for galectin-3 (p<0.001). In PT, low-grade lesions displayed a similar distribution of galectin-3 reactive tumors as the juvenile FA (40.5%). Intermediate and high-grade lesions were highly positive for galectin-3 (88.9 and 100%, respectively). Thus, there was a positive correlation between galectin-3 accumulation and histological grade in PT (p < 0.001). Stromal cells from PT also stained positively for TP53, VEGF and NO synthase 3. A significant accumulation of all these three markers was observed in higher grade lesions as compared to intermediate and low grade lesions (accumulation of TP53, p= 0.036; accumulation of VEGF, p= 0.001, accumulation of NO synthase 3, p< 0.001). Concomitant analysis of galectin-3 and the different markers analyzed showed that galectin 3 accumulation was associated with (1) stromal cell TP53 status; (2) NO synthase expression. In vitro, galectin-3 transcription is tightly controlled by normal p53; mutations in p53 are associated with the relaxation of this control; and, galectin-3 protects cells from NO induced cell death. Galectin-3 associated with high histological grade PT and could be used as a surrogate marker for p53 status, discriminating potentially more aggressive forms of fibroepithelial tumors.

668 Secretory immune system control of estrogen-responsive breast and other target tissue cell growth in culture.

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Background: We have identified new methods for demonstrating estrogen mitogenic effects in serum-containing cell culture (DA Sirbasku & JE Moreno, *In Vitro Cell Dev Biol* 36:428-446; 2000). These studies show that serum contains new estrogen-reversible inhibitors. Our goal was to establish the molecular identities of these inhibitors and to demonstrate their activity in their purified forms in serum-free defined culture medium.

Materials and Methods: The cell lines used were all estrogen growth responsive *in vivo* and *in vitro*. They included MCF-7, ZR-75-1, T47D, MTW9/PL2 rat mammary, GH rat pituitary, and Syrian hamster H-301 kidney cell lines. The serum-free defined media and growth assay methods are described in the cited report. The serum inhibitors were purified by a two-step cortisol-agarose and phenyl Sepharose chromatography method and identified as polymeric/dimeric IgA (pIgA) and pentameric IgM. Commercial pIgA and IgM were equally effective as estrogen-reversible inhibitors.

Results: All cell lines replicated at near maximum rates in defined media; estrogens had little additional effect. Addition of 10 to 20 µg/mL of pIgA or IgM (i.e. low nM levels) in the absence of estrogens completely arrested growth. Adding 1-100 pM 17β-estradiol reversed the inhibition and restored optimum growth. Estrogen caused 8 to 64-fold increases in cell number. IgG and TGFβ did not substitute for pIgA/IgM. The effects of pIgA and IgM were not species specific. This analysis, plus that of the activity of the secretory form of IgA (sIgA), indicates that the effect of pIgA resides in the Fc domain rather than in antigen binding domains.

Discussion: These studies put forward the concept that the two major secretory immunoglobulins produced by B cells in the lamina propria of breast tissue act as paracrine negative regulators of adjacent epithelial cells. There is no previously published role for the secretory immune system in breast cancer cell growth. Because breast B cells originate from gut mucosal Peyer's patches, and are targeted to breast by hormones, new conceptual aspects of breast cancer etiology, treatment and prevention are raised. This includes new diagnostic and prognostic tools, new immune therapies for early stage disease, an entirely new concept of natural immune risk reduction and ultimately, application to eradication of breast cancer by oral immunization.

669 Mechanism by which AP-1 blockade inhibits growth and suppresses G1 cyclin expression in breast cancer cells.

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The AP-1 transcription factor plays a critical role in signal transduction pathways that convey the extracellular growth signals to the nucleus, leading to the expression of genes associated with malignant transformation in many cells including breast cancer cells. We have previously shown that blockade of AP-1 activity by over-expressing a dominant negative form of cJun (cJunDN, Tam67) inhibited growth factor-induced breast cancer cell growth *in vivo* and *in vitro* . We hypothesize that this growth inhibition is the result of down-regulation of cell cycle regulators causing a cell cycle block. In the present study, we examined cyclin D and E expression levels in cells that express cJunDN and investigated the mechanism by which AP-1 blockade suppresses breast cancer cell growth. We used clones of MCF7 breast cancer cells expressing Tam67 under the control of an inducible promoter. Using these cells we examined the effect of AP-1 blockade on cell growth with cell proliferation assays, ^{3}H-thymidine incorporation assays, and flow cytometry. We observed that Tam67 expression inhibited breast cell growth in the presence of serum. The inhibition was caused by a cell cycle block in the G1 phase. Expression of cJunDN was associated with reduced cyclin D and E expression as determined by Western blotting. We are now examing the mRNA levels for both G1 cyclins at all time points in synchronized MCF7 cells expressing Tam67, and are also examing promoter activity for D and E cyclins in the presence and absence of AP-1 blockade. Previous studies focusing on the effect of Tam67 expression on growth factor signaling revealed that AP-1 is critical to transduce hormone-induced mitogenic signals in breast cancer cells. We now demonstrate that the growth inhibition is due to a G1 cell cycle block caused by decreased cyclin D and E expression. These findings suggest that decreased expression of cyclins D and E leads to effective growth suppression of normal and malignant breast cells, and that specific inhibitors that mimic the effect induced by Tam67 could be used as potential agents for treatment and prevention of breast cancer.

670 Upregulation of erbB2/erbB3 and enhanced signal transduction via the phosphatidylinositol 3-kinase pathway in a tamoxifen-resistant breast cancer cell line.

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Background: Breast cancer cells initially sensitive to the antioestrogenic drug tamoxifen can develop resistance to its actions following prolonged exposure. It is evident that alternate signalling mechanisms may become activated in order for cell growth to occur in the presence of the drug. We have investigated the differential expression of several key growth factor receptors and their intracellular signalling pathways (MAP kinase and PI 3-kinase) in a tamoxifen-resistant MCF-7 cell line (Tam^{R}-1) in comparison with parental tamoxifen-responsive wild-type (WT) cells.
Materials and Methods: Cell lysates of WT and Tam^{R}-1 cells were prepared from exponentially growing cultures. Equal quantities of protein were electrophoresed and immunoblotted using antibodies against several growth factor receptors (phospho-EGFR, phospho-erbB2, erbB3 and erbB4), intracellular signalling molecules (phosphorylated Raf, MEK1/2, ERK1/2, p90rsk and Akt) and activated oestrogen receptor (phosphorylated ERα ser^{118} and ERα ser^{167}).
Results: There was an increase in levels of phospho-erbB2 and erbB3 in Tam^{R}-1 cells compared with WT. No differences in levels of phospho-EGFR or erbB4 were observed. Levels of phosphorylated Akt, ERK1/2, p90rsk and activated ERα ser^{167} were also higher in Tam^{R}-1 cells than in WT. No differences in levels of phospho-ERα ser^{118} or other components of the signalling pathways were detected.
Discussion: The data suggests a novel mechanism by which tamoxifen-resistant cells may circumvent ligand-dependent growth in the presence of tamoxifen. Unlike previous reports, this does not involve an upregulation of EGFR but rather a possible heterodimerization of erbB2 with erbB3. The lack of changes in ERα ser^{118} phosphorylation or in most of the components of the MAP kinase pathway indicate that this signalling cascade plays only a minor role in these Tam^{R}-1 cells. However, increased levels of phosphorylated Akt, ERK1/2 and p90rsk suggest that ligand-independent activation of the oestrogen receptor possibly via phosphorylation of ERα ser^{167} is likely to be involved in growth in the tamoxifen-resistant phenotype.
(Supported by the Breast Cancer Campaign)

671 The role of activin signal transduction in breast cancer.

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Background: The protein hormone activin is a member of the TGF-β superfamily of pleiotrophic growth factors. Activin exerts growth regulatory action throughout the body axis, and has been associated with mouse mammary gland development and human breast carcinogenesis. We hypothesize that the protein components involved in the activin signal transduction cascade will be dynamically regulated in human mammary gland carcinogenesis, and that the loss of activin signaling is associated with high grade malignancy.**Materials and Methods:** Paraffin-embedded tumor sections from grades 1-3 breast cancer were utilized for the immunohistochemical detection and cellular localization of activin subunits (βA and βB), receptors (ActRIIA, ActRIIB, and ActRIB), and cytoplasmic co-activators (Smads 2, 3, and 4). Immunohistochemical scoring was based on a scale of 0 (absent) to 3+ (very intense), and was verified by a cytopathologist. Immunofluorescence was used to detect the nuclear co-localization of Smads 2 and 3. Proliferating cell nuclear antigen, PCNA, staining was also studied to correlate cell proliferation with the activin signaling protein components. Additional clinical and prognostic data was available for correlative analysis.**Results:** Activin βA subunit immunoreactivity was consistently very intense for cancers of all 3 grades. Activin βB subunit immunoreactivity was present for cancers of all 3 grades. Immunoreactivity for activin receptors decreased as tumor grade increased. Activated nuclear Smad3 immunoreactivity was more intense in grade 1 cancers, while cytoplasmic Smad3 immunoreactivity was more intense in grade 3 cancers. This finding was confirmed by immunofluorescence. Smad2 and Smad4 immunoreactivity also decreased as tumor grade increased. As expected, nuclear and cytoplasmic PCNA immunoreactivity was greater as tumor grade increased.**Discussion:** Intact activin signal transduction has been associated with inhibition of cell proliferation through the induction of G1 phase cell cycle arrest. Using immunohistochemistry and immunofluorescence, we have shown that the activin signal transduction cascade receptors and nuclear Smads are decreased as tumor grade and cell proliferation increase. These findings indicate that alterations in the activin signaling pathway may lead to differences in the governance of growth in breast cancer tissue.

672 The expression of the hepatocyte growth factor regulatory system in breast cancer.

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Background: Hepatocyte growth factor (HGF) stimulates tumour cell-cell interactions, matrix adhesion, migration, invasion and angiogenesis. HGF is produced as an inactive precursor called pro-HGF. Pro-HGF requires proteolytic conversion, by HGF activator (HGFA), to evoke a biological response. The two HGFA inhibitors, HAI-1 and HAI-2, inhibit the generation of biologically active HGF, through their interaction with HGFA. This study determined the expression of the HGF regulatory system, HGFA and its inhibitors, along with HGF and its receptor (c-Met), in breast cancer.
Materials and Methods: Breast cancer tissue (n = 100) and normal background tissue (n = 20), was obtained immediately after surgery. The median follow-up for the patients was 72 months. HGF, c-Met, HGFA, HAI-1 and HAI-2 expression was quantified using real-time quantitative PCR.
Results: Breast cancer specimens expressed a statistically higher level of the molecules, than that of the normal breast tissue (HGF-5.2 vs 48.8 copies/µl; c-Met-9339.4 vs 22155.5 copies/µl; HGFA-12.9 vs 46.3 copies/µl; HAI-1-450.3 vs 960.1 copies/µl; and HAI-2-114.6 vs 438.7 copies/µl). Tumour tissues from node positive patients expressed a higher level of HGFA (59.4 copies/µl) than from the patients without nodal involvement (38 copies/µl). Interestingly, HAI-2 was expressed to a lower degree in positive nodes (341.3 copies/µl), than that of the node negative breast cancer tissues (440.9 copies/µl). In addition, upon comparison of TNM classification groups, HAI-2 was also found to be statistically lower in the TNM 3 breast cancer group when compared to TNM groups 1 and 2 ($p<0.001$), thus associated with a poor prognosis.
Discussion: This study has shown that there are aberrant levels of HGF, c-Met, HGFA, HAI-1 and HAI-2 expressed in breast cancer tissues. The levels of HAI-2 are inversely correlated with nodal involvement. The HGF regulatory system may therefore have an important role in the progression of breast cancer.

673 Interleukin-7 is a putative lymphangiogenic factor in endothelial cells.

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Introduction:
The formation of new lymphatic vessels in tumour, tumour lymphangiogenesis, is known to increase and associate with local and nodal metastasis in breast cancer. However, there is very little knowledge on the factors that may induce lymphangiogenesis (lymphangiogenic factors). Here, we studied the effects of a range of cytokines/growth factors on the process of lymphangiogenesis.
Methodology:
Human endothelial cells (HECV) were treated with cytokines over a specified period and a range of concentrations. Cell growth was measured using a MTT assay. Expression of lymphatic specific markers (LYVE1, podoplanin, and Prox-1), a vascular endothelial marker VEGF R2, and cytokine receptor was determined using RT-PCR and quantitative RT-PCR. β-actin was used as the internal house keeping gene.
Results:
Of the cytokines tested, interleukin-7 was found to significantly increase the expression of lymphatic markers in HECV cells, as shown by both qualitative and quantitative RT-PCR. However, IL-7 did not affect the level of VEGF R2. The time course study revealed an enhanced expression of Prox-1, LYVE-1, and podoplanin in cells treated with IL-7, with maximum effects seen between 2-4 hours after treatment. In addition, HECV cells expressed high level of IL-7 receptor, but not endogenous IL-7, as revealed by RT PCR. The growth of the HECV cells was seen to increase in the presence of IL-7, as demonstrated by the MTT assay. The optimal concentrations of IL-7 at which the cells exhibited growth were found between 0.6 and 2.5ng/ml.
Conclusion:
This is the first study to show that IL-7 has an impact on the process of lymphangiogenesis as it enhances the expression of lymphatic endothelial markers in human endothelial cells. This may have an important influence on the process of lymphangiogenesis in human breast cancer.

674 mRNA expression of VEGF-C and VEGF-D in breast cancer: association with lymph node metastasis.

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Background: Lymph node metastasis is a major prognostic factor for breast cancer patients. Vascular endothelial growth factor (VEGF) -C and VEGF-D, as ligands for VEGF receptor-3, are capable of stimulating lymphangiogenesis and at least VEGF-C can enhance lymphatic metastasis. The aim of the present study was to explore that VEGF-C and/or VEGF-D mRNA expression correlate with lymphatic invasion and lymph node status in breast cancer.
Materials and Methods: Human breast tissues were obtained from 34 breast cancer patients. Total RNAs were isolated from 34 surgical specimens of breast cancer tissue and 7 normal breast tissues. The relative mRNA abundance of VEGF-C and VEGF-D was measured by real time reverse transcription-PCR analysis based on TaqMan method and the results were standardized with GAPDH mRNA expressions. Statistical analyses were performed using Mann-Whitney's U test and the statistical significance was defined as $p<0.05$.
Results: VEGF-C mRNA expression was detected in both breast cancer and normal breast tissue without statistical significance. There was no significant relationship between VEGF-C mRNA level and lymphatic invasion or lymphnode status. VEGF-D mRNA expression was significantly decreased in breast cancer tissues compared to normal breast tissue ($p<0.01$). and was significantly decreased in cancer tissues with vs without lymphatic invasion ($p<0.01$). VEGF-D mRNA levels were also decreased in cancer tissues of node positive cases, although not significant ($P=0.059$). However, the value of VEGF-C/VEGF-D was significantly increased in breast cancer tissues compared with normal breast tissues ($p<0.05$). VEGF-C/VEGF-D was also significantly increased in breast cancer tissues of node positive patients compared with node negative patients ($p<0.05$).
Discussion: Our results suggest that the increased VEGF-C/VEGF-D mRNA expression ratio may have associations with tumorigenesis and/or lymph node metastasis in breast cancer.

675 Growth inhibition in human breast cancer cells with the combination of a PKC inhibitor and zoledronic acid.

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Background: Protein kinase C (PKC) isoenzymes are anti-apoptotic and involved in neoplastic cell survival. Reduction of PKC activity initiates apoptosis and enhances the cytotoxic effects of a variety of chemotherapeutic agents. Zoledronic acid, a new generation bisphosphonate used in the treatment of cancer-induced bone metastases, significantly reduces cell number and induces apoptosis in human breast and prostate carcinoma and multiple myeloma. The purpose of this study was to assess the effect of combining a PKC inhibitor with zoledronic acid on human breast cancer cell growth.
Materials and Methods: The effect of combining a PKC inhibitor (PKC412) and zoledronic acid compared to either agent alone was tested in control transfected (MCF/neo) and HER-2/neu transfected (MCF/18) human breast cancer cell lines grown in media supplemented with 10% fetal bovine serum. Cell number was determined after a 3 day incubation using the MTT tetrazolium dye assay.
Results: Treatment with the PKC inhibitor (0.1 -5 uM) resulted in dose-dependent growth inhibition in both cell lines. The control MCF/neo cells were less sensitive to the inhibitor (IC50: 3.8 uM) than the HER-2/neu overexpressing MCF/18 cells (IC50: 0.8 uM). Treatment with zoledronic acid (1-10 uM) also gave dose-dependent growth inhibition. The HER-2/neu MCF/18 cells, however, were less sensitive to zoledronic acid (IC50: 9.3 uM) than the MCF/neo cells (IC50: 6.0 uM)). The combination of zoledronic acid (5 uM) and the PKC inhibitor at various concentrations appeared to have an enhanced inhibitory effect on both cell lines.
Conclusion: The bisphosphonate, zoledronic acid, and the PKC inhibitor each produced dose-dependent growth inhibition as single agents in both a HER-2/neu transfected human breast cancer cell line (MCF/18) and a control vector transfected line (MCF/neo). The MCF/neo cells were less sensitive to the PKC inhibitor while the MCF/18 cells were less sensitive to zoledronic acid. The combination of zoledronic acid and the PKC inhibitor gave an enhanced inhibitory effect on both cell lines compared to either agent alone.

676 Tissue-type plasminogen activator (tPA) is upregulated in metastatic breast cancer cells exposed to insulin-like growth factor-I (IGF-I).

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Background: High serum IGF-I in breast cancer patients correlates with poor outcomes. Mechanisms by which IGF-I contributes to invasiveness remain to be defined. We have reported that MDA-MB-435s breast cancer cells express tPA, but not urokinase type plasminogen activator. Decreased expression of the type I insulin-like growth factor receptor (IGF-IR) resulted in lower levels of tPA mRNA. In this study we examined the role of IGF-I in regulating tPA in parental MDA-MB-435s cells and in cells carrying an antisense IGF-IR construct.
Material and Methods: Northern blot analysis with a tPA cDNA hybridization probe was used to assess the level of tPA expression in the cells (parental, antisense IGF-IR and control transfected) treated with 10 ng/ml of IGF-I. An ELISA kit was used for quantification of tPA protein. Matrigel assays were performed to determine the cellular invasiveness of cells treated with IGF-I (20ng/ml).
Results: We observed a significant increase in tPA mRNA over time with a peak at 8 hrs that remained elevated for more than 24 hrs in cells treated with IGF-I. tPA protein was also elevated by IGF-I treatment in a time dependent manner. Cells treated with IGF-I were significantly more invasive than control cells. tPA mRNA levels were elevated in antisense IGF-IR cells exposed to IGF-I, however the level of tPA mRNA in the control cells was slightly higher. The tPA protein level was much lower in the cells carrying the antisense IGF-IR construct compared to the control cells.
Discussion: Studies have shown that tPA plays a major role in cellular invasion for glioblastoma, pancreatic and melanoma tumor cells. Out data incicate that tPA plays a similar role in certain human breast cancers. This is the first report that demonstrates that IGFs may function with tPA in a synergistic manner to facilitate tumor invasion in breast cancer.

677 Use of a novel breast tissue explant culture system as a model to investigate breast tumorigenesis.

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Background: The growth hormone/insulin-like growth factor-1(GH/IGF-1) axis has long been implicated in the development of breast cancer. Previously we have shown the expression of GH, IGF-1and ghrelin, an endogenous GH secretagogue, in normal and malignant breast tissue. *In vitro* studies have examined the effects of these hormones on breast tissue cell lines. However these are not physiologically representative of the human breast and may not reflect normal tissue responses.

Aims: (1) To develop a method for the culture of normal and malignant breast tissue explants and demonstrate prolonged cell viability.

(2) To use this system to investigate the effects of ghrelin on the local expression of GH/IGF-1 mRNA and that of tumour associated genes, PCNA and c-myc.

Methods: Fresh normal and malignant breast tissue was collected from surgical specimens (n=5). Full patient consent and ethical approval was obtained in all cases. 10-20mg samples were cultured in serum-free media for up to 96hrs. Tissue viability was assessed using MTS assay, and cell death was assessed by measuring LDH release into the media. Subsequent experiments involved the addition of ghrelin to breast tissue samples that were incubated for 4 to 24 hours. mRNA levels of GH, IGF-1, PCNA and c-myc genes were quantified using the RT-PCR 'Taqman' assay.

Results: The MTS assay showed both normal and malignant tissue explants to be viable for up to 96 hours. LDH levels remained stable throughout the 96 hours, being less than 50% of the total LDH release obtained with tissue lysis (Triton). The addition of ghrelin had no effect on the expression of GH mRNA levels. In two of the normal samples there was downregulation of IGF-1 mRNA levels. The effects on PCNA and c-myc mRNA levels were variable amongst individual patients.

Conclusion: We have established and validated a novel system for the culture of fresh normal and malignant breast tissue and shown that it can be used to investigate breast tumourigenesis. The effects of ghrelin indicate marked interpatient variability and the requirement for a larger numbers of samples.

678 The expression of insulin-like growth factor 1 is down regulated in invasive ductal carcinoma in comparison with adjacent non-cancerous tissue.

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Background

Insulin-like growth factor 1 (IGF-1) is important in normal cellular growth of the breast and is a non-hormonal survival signal for breast cancer. The effects of IGF-1 are mediated through the tyrosine kinase IGF-1 receptor (IGF-1R). Increased circulating levels of IGF-1 are associated with an increased risk of breast cancer. IGF-1 is thought to exert its actions on the breast via an endocrine and autocrine/paracrine route. The question of the relative importance of circulating versus endogenous production has not yet been resolved. Endogenous production of IGF-1 and IGF-1R in both the stromal and epithelial compartments of breast tissue support the autocrine/paracrine theory.

This study investigated the expression of IGF-1 and IGF-1R mRNA in invasive ductal carcinoma (IDC) and adjacent non-cancerous tissue (ANCT).

Method

31 patients with IDC were randomly selected. Paired samples of tumour and ANCT were homogenised; RNA isolated and cDNA synthesised. mRNA levels were measured using a real-time PCR machine.

The relative levels of mRNA expression were calculated compared to a housekeeping gene (ribosomal 18s). The association between mRNA in cancerous and ANCT was calculated using a paired t test.

Results

The median values of relative expression of IGF-1 were 0.469 (0.281-0.711) and 0.762 (0.338-0.874) in tumour and ANCT respectively. The IGF-1 receptor values were 0.118 (0.076-0.169) in tumour and 0.094 (0.036-0.124) in ANCT.

The p-value for IGF-1 was 0.0002 indicating a significantly lower level of IGF-1 in the tumour specimens. 27/31 samples had higher IGF-1 levels in ANCT.

No signficant difference was detected between the samples for IGF-1R.

Conclusion

IGF-1R showed a universal expression throughout the normal and IDC samples. This supports the hypothesis that IGF-1 acting via the IGF-1R plays a role in normal and cancerous tissue.

The significantly lower levels of IGF-1 in tumour samples in comparison with the ANCT suggests that the relative importance of the paracrine/endocrine role of IGF-1 varies. In normal breast tissue the importance of paracrine production is demonstrated. Endocrine factors appear to predominate in IDC however the relative importance of endocrine production in normal breast tissue is not defined.

679 The correlation between two methodologies used for detecting the levels of insulin-like growth factor 1 receptor in breast tumours.

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Background

Insulin-like growth factor 1 (IGF-1) plays an important role in normal cellular growth and development of the breast and is also a non-hormonal survival signal for breast cancer. The effects of IGF-1 are mediated through the IGF type 1 receptor (IGF-1R), a tyrosine kinase receptor. A threshold level of IGF-1R is required for cells to demonstrate a mitogenic response to IGF-1. In primary breast tumours the IGF-1R is over expressed and hyperphosphorylated. A large range of receptor levels have been reported, from 39-93%. Studies examining IGF-1R levels in relation to prognosis have yielded conflicting results and the methodology used may explain these differences.

In breast tissue IGF-1R has been measured by ^{125}I-IGF-1 ligand-binding assay based techniques. Immunohistochemical (IHC) methods using various antibodies to the IGF-1R, commonly the alpha sub-unit, allow a more visual display of localisation of the receptor. The recent advance in real-time PCR (RTPCR) has allowed very sensitive measurement of IGF-1R mRNA levels. The present study aims to examine the correlation between RTPCR and IHC for IGF-1R measurement.

Method

Tumour samples (n=15) were homogenised; RNA isolated and cDNA synthesised. mRNA levels were measured using an RTPCR machine.

Frozen tumour samples were sectioned and mounted on microscope slides. After peroxidase blocking the slides were incubated with 20µg/ml of the IGF-1R monoclonal antibody αIR3. Following standard IHC the immune complex was visualised using DAB.

Slides were scored for IGF-1R staining (0=no staining, 3=strongest staining) and for the percentage of cells with positive staining in 2 visual fields by 2 observers.

Analysis of variance was used to examine the relationship between mRNA expression and IHC levels of IGF-1R.

Results

No correlation was detected between the mRNA levels for IGF-1R and the strength of IHC staining (p=0.399). There was also no relationship with the percentage of cells staining positively for IGF-1R (p=0.786).

Conclusion

In established breast cancer the levels of IGF-1R mRNA expression do not seem to be correlated with the IHC levels of receptor. This may be attributable to a variety of factors. The most significant of these could be that both techniques are measuring different steps along the pathway of IGF-1R production.

680 Downregulation of Grb2 protein expression led to inhibition of KGF-induced motility in MCF-7 breast cancer cells.

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Background: The metastasis of breast cancer is known to be directly associated with the motility of breast cancer cells. In previous studies we reported that keratinocyte growth factor (KGF) enhanced the motility of estrogen receptor-positive breast cancer cells (Zang, XP, et al., Clin. Expt. Metastasis 18:573, 2001) and increased the expression of GRB2 mRNA (Zang, XP, et al., Breast Cancer Res. Treat., 58: 110, 2000). Since Grb2 is involved the recruitment of signal transduction mediators, we examined the effects of Grb2 on KGF-mediated signal transduction and cell motility in MCF-7 cells.

Materials and Methods: MCF-7 cells were treated with liposomal Grb2 antisense (L-Grb2) or liposomal control (L-control) oligos and stimulated with human recombinant KGF (50 ng/ml). A cell wounding assay was used to examine cell proliferation and motility at 24 and 48 h following treatment.

Results: L-Grb2, but not L-control, decreased KGF-mediated MCF-7 cell proliferation and motility. We postulate that Erk1,2 and Akt may be potential downstream signaling proteins involved in KGF-mediated motility. KGF was found to increase phospho-Erk1,2 and phospho-Akt levels in MCF-7 cells. In addition, L-Grb2 decreased both phospho-Erk1,2 and phospho-Akt levels in KGF-stimulated cells.

Discussion: These results indicate that downregulation of Grb2 expression inhibits KGF-stimulated breast cancer cell motility, which is correlated with inhibition of Erk1,2 and Akt activation. Thus, Grb2 appears to play a vital role in the regulation of KGF-induced motility by activating Erk1,2 and Akt signaling.

This study was supported in part by grants to JTP [NIH/NCI (CA-89740) and DOD (DAMD17-01-1-0591)].

681 Downregulation of interleukin 6 expression results in growth inhibition of MCF-7 breast cancer cells.

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Interleukin 6 (IL-6) plays an improtant role in the neoplastic process through its action on cancer cell adhesion, motility, proliferation, tumor-specific antigen expression, and thrombopoiesis. IL-6 exerts its activity by binding to the IL-6 receptor (IL-6R). In the present study, MCF-7 breast cancer cells were cultured with human IL-6 protein. Also, MCF-7 cells were treated with either a specific polyclonal antibody to human IL-6 or synthetic antisense oligonucleotides (ODNs) targeted to IL-6 and the IL-6R. Cell growth was measured, and we found that human IL-6 protein did not significantly increase the proliferation of MCF-7 cells. However, the IL-6 produced by MCF-7 cells can be bound by rabbit anti-human IL-6 antibody, and this resulted in a significant dose-dependent inhibition of cell proliferation. IL-6 and IL-6R antisense ODNs caused a marked and specific decrease in IL-6 and IL-6R mRNA and proteins. Both IL-6 antisense ODN and IL-6R antisense ODN significantly inhibited the proliferation of MCF-7 cells. However, exogenous addition of IL-6 can partially reverse the growth inhibition caused by IL-6 antisense ODN but not the inhibition caused by IL-6R antisense ONDs. Also, in the presence of equal molar concentrations of IL-6 or IL-6R antisense ODNs, IL-6R antisense ODN was a more effective inhibitor of proliferation than IL-6 antisense ODN. In conclusion, IL-6 plays an improtant role in maintaining the growth of MCF-7 breast cancer cells. These results suggest that careful modulation of IL-6 expression of cells may be a beneficial breast cancer therapy.

682 The selective growth of bone-metastatic breast carcinoma cells by interlukin-3 (IL-3) is not mediated by STAT5a/b.

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Background: The molecular mechanisms by which breast carcinoma (BRCa) cells selectively grow in the bone marrow are unknown. We had previously shown that IL-3 enhances the selective growth of bone-metastatic BRCa cells in vitro, and that bone-metastatic BRCa cells significantly over express the a-chain of the IL-3 receptor. IL-3 induces the growth of hematopoietic cells through the Jak2/Stat5 signaling pathway. We hypothesize that IL-3 induces the growth of bone-metastatic BRCa cells by activation of the Stat5a/b proteins.

Materials and Methods: We obtained protein from unstimulated and IL-3 stimulated (50 Ng/ml) (24-48 hrs.) pleural- (MDA-231) and bone-metastatic BRCa cells. Western blots were performed to detect Stat5a/b and pStat5 (Tyr) levels. The images were analyzed by densitometry. Statistical significance was established at $p < 0.05$.

Results: 1) Unstimulated bone-metastatic BRCa cells showed a significantly higher expression of Stat5a compared to the pleural-metastatic cell, 2) exposure of the bone-metastatic BRCa cells to IL-3 did not increase the expression or the tyrosine phosphorylation of Stat5a, 3) none of the cell lines tested expressed the Stat5b protein.

Discussion: Our results demonstrated that the intracellular mechanism by which IL-3 induces the selective growth of bone-metastatic BRCa cells in the bone marrow do not involve increased expression or phosphorylation of the Stat5a/b signaling pathway. These findings suggest that bone-metastatic BRCa cells utilize other signaling pathways in their growth response to IL-3.

683 MMTV-related sequence expression in human mammary carcinoma.

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***Introduction*:** Existence of retroviruses responsible for human mammary carcinoma (MC) induction is under discussion for many years, it numbers dozens of publications. However, no cogent arguments have been presented to date. In favor of possible involvement mouse mammary tumor virus (MMTV) -related agent into human mammary carcinogenesis we have found the following:

Results:

1. Antibodies recognizing MMTV structural proteins (the most specific env gene product - gp 52) were detected in 70-72% of MC patients in comparison to 3-5% of the patients with other tumors and healthy blood donors.
2. An antigen immunologically analogous to MMTV was detected not only in MC and blood sera, but in peripheral blood lymphoid cells of MC patients and their relatives, too. Notably, its expression in T-cells occurred with the incidence about 100% only within these two groups. The antigen expression in B-cells could be detected also in control groups with the incidence not more than 25-32%.
3. About 75% of mammary carcinoma (MC) patients are positive by expression of MMTV -related sequences in peripheral blood lymphocytes (PBL). The expression is correlated with presence of human T-cell specific antigen immunologically related to gp52. The sequence frequency in control groups - donors and patients with gynaecological tumors - is statistically lower ($p<0.01$).
4. Using PCR of PBL DNA and primers for gp52 coding area of the env MMTV gene, specific PCR-products corresponding to the env MMTV gp 52 region have been revealed in 75% of DNA samples from MC patients, in 29,7% of donor and in 18,7% of gynaecological patients. Sequencing of specific 650bp PCR and 700, 280 bp RT-PCR products has shown about 95-97% homology with gp52 -coding area of exogenous env MMTV from C3H mouse strain. One from the specific PCR products of 927 bp long was mapped on metaphase chromosomes of MC patients using biotin labeled probe, showing a primary sites on chromosomes 1,5,8,10,11,13, 15 from PBL of MC patients.

***Summary*:** The env MMTV-related sequences in PBL genome have likely exogenous origin. They might be considered as a molecular MC marker. Its occurrence in PBL is indicative of MC risk.

684 Increasing evidence for a human breast cancer virus.

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Background: In 1984, we obtained the first evidence of apparent MMTV-like antigens in Tunisian breast cancer patients as part of a multidisciplinary investigation of a rapidly progressing breast cancer (RPBC) seen with high frequency there. The apparently specific gp-52 antigen was seen only in tumor cells and the reaction was blocked by specific anti-gp 52 antisera. The purpose of this project was to confirm by molecular techniques the possible role of a human virus related to MMTV in the pathogenesis of human breast cancer.

Methods: 39 paraffin blocks, previously used in clinical and immunologic studies, were selected for detecting a MMTV-like *env* 250-bp sequence from breast cancer patients seen and treated at the Institut Salah Azaiz in Tunisia. Relationships between the presence or absence of the MMTV-like *env* gene sequence and individual clinical, histological or hormonal parameters were compared using Fisher's exact test. Disease-free interval comparisons between MMTV-*env* positive and negative cases were tested for statistical significance using life table analysis. A second laboratory studying MMTV sequences was given 24 biospecimens under code to determine comparability with the first testing laboratory.

Results: Of the 38 cases analyzed, 28 (73.7%) tested positive for the 250-bp sequence of the MMTV-like *env* gene. Comparisons to recent studies that had examined breast cancer cases from the U.S. and Italy for the presence of the 250-bp sequence using the same amplification techniques in the same laboratory found Tunisian cases to have a significantly higher proportion of *env*-positive cases than cases from the U.S. (30-40%) or Italy (37%). Also, when comparing PEV-positive cases with PEV-negative cases, a higher proportion of aggressive tumors showed the *env* sequence ($p=0.06$). There was 100% concordance between the two laboratories in regard to positive and negative results. **Conclusion**: This study supports early immunologic studies indicating a significantly higher proportion of Tunisian breast cancer patients with infection by a MMTV-like virus compared to patients in the U.S. and italy. There also appears to be a relationship between cancer aggressiveness and possibly inflammatory breast cancer, which is far more common in Tunisia than the U.S. Finally, the concordance between two laboratories receiving biospecimens under code indicates that the virus-related sequences can be detected reproducibly. Their precise nature and significance remain to be determined.

685 Human papillomavirus (HPV) DNA detection by polymerase chain reaction (PCR) in breast cancer: preliminary results.

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Background : The involvement of HPV in the pathogenesis of anogenital tumors, particularly cervical cancer, is well documented. In contrast, after several studies, the association between HPV and breast cancer remains an issue under ongoing debate. The purpose of this study is to assess the presence of HPV-PCR in core-biopsy proven breast cancer patients with no history of previously known HPV exposure. **Material and Methods** : by polymerase chain reaction (PCR) we investigated preliminarily 51 of 110 specimens of breast cancer tissue in paraffin blocks using specific primer E6 for HPV 16 and 18. We also analyzed the correlation between HPV DNA expression and main prognostic factors for breast cancer outcome. Patients also had cervical smear collected at the same time of breast core biopsy. **Results** : HPV 16 DNA was detected in 26% of the tumors. There was no significant association between HPV and axillary lymph node status, tumor size, histologic subtype, nuclear grade or hormone receptor status. Up to now no cervical displasia or invasive carcinoma was detected. **Discussion** : our partial results are in line with previous studies using specific primers and suggest that HPV infection can play a role in the pathogenesis of breast cancer in patients. Further conclusions and definitive statistical analysis will be made after investigation of the remaining specimens.