tissue sections of representative areas in all cases. We counted two hundred nuclei and estimated the percentage of nuclei with split signal, normal signal, and the other various types of abnormal signals.
Results: Six cases of seven PLS showed CHOP split signal ranged 0.5 to 3% of counted nuclei, while all cases of MLS/RC exhibited CHOP rearrangement over than 10% of counted nuclei. All cases of PLS showed various distribution of normal and abnormal signals in each histological areas.
Conclusions: A CHOP rearrangement in PLS should be recognized as only a representative part of complex karyotypic feature, because the number of cells with split signals was surely minute compared with that of MLS/RC, and the signals were found in any areas despite of its histological difference. Therefore, we have to carefully estimate the association between the results of FISH analysis and histological subtypes with a characteristic rearrangement.

68 The Metastatic Dichotomy between Sarcomas and Carcinomas Is Partially Explained by Their Relative Levels of Vasculogenesis and Lymphangiogenesis but Not by Their VEGF Expression Profile

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Background: Human carcinomas are known to metastasize primarily through lymphatics to lymph nodes whereas sarcomas with few exceptions spare the lymphatics and metastasize hematogenously to visceral organs. However the reason for this metastatic dichotomy is not understood. With the newly emerging experimental evidence that metastasis may be regulated by local vasculogenesis *v* lymphangiogenesis, we wondered whether these might differ in sarcomas *v* carcinomas and account for their metastatic dichotomy.
Design: We decided to examine 20 sarcomas and 20 carcinomas to assess their comparative degrees of vasculogenesis *v* lymphangiogenesis. We conducted IHC studies with lymphatic (D2-40), vascular (CD31) and proliferation (Ki-67) markers singly and in combination, the latter employing a dual chromogen technique designed to determine the relative levels of vasculogenesis *v* lymphangiogenesis and vascular *v* lymphatic invasion. We also studied the expression profile of the vascular endothelial growth factor (VEGF) family members: VEGF-A, -B, -C, -D in these tumors by RT-PCR and real time PCR.
Results: In all the sarcomas and carcinomas studied, four vascular populations were in evidence: D2-40 lymphatics with and without tumor emboli; CD31 blood vessel capillaries with and without tumor emboli. The D2-40 lymphatics were more numerous in the carcinomas ($p<.001$) and the CD31 blood vessels were more numerous in the sarcomas ($p<.01$). The sarcomas showed more vascular invasion ($p<.01$) than the carcinomas which exhibited greater lymphatic invasion ($p<.01$). While a greater percentage of the carcinoma D2-40 lymphatics showed proliferation ($p<.01$), a greater percentage of the sarcoma CD31 vessels showed proliferation ($p<.05$).VEGF-A, -B, -C and -D transcripts were detected by RT-PCR in all of the sarcomas and carcinomas but by real time PCR the sarcomas surprisingly had 25-50 fold greater VEGF-C whereas the carcinomas had 3 fold greater VEGF-A. VEGF-B, -D were low in both groups.
Conclusions: These studies suggest that sarcomas, in contrast to carcinomas, stimulate minimal lymphangiogenesis but maximal vasculogenesis. But this effect is not mediated by the relative levels of their VEGF family members because VEGF-C which is thought primarily to stimulate lymphangiogenesis is paradoxically high in sarcomas This suggests that the mechanism behind the differential vasculogenesis / lymphangiogenesis in sarcomas needs to be elucidated.

69 Differential Expression of the Oncoprotein c-Jun in Liposarcomas Highlights Tumors of Dedifferentiated Type

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Background: A recent report demonstrated that c-Jun amplification and overexpression is a distinguishing characteristic of a subset of aggressive sarcomas, which appear to represent dedifferentiated liposarcomas (DDLPS). Overexpression of c-Jun was also shown to block adipocytic differentiation in a model system (Mariani et al., Cancer Cell 2007). However, only limited data are available on the relative levels of c-Jun protein in liposarcomas with both well-differentiated (WD) and dedifferentiated (DD) components.
Design: We performed immunohistochemistry for c-Jun in a series of liposarcomas (DDLPS, well differentiated (WDLPS), myxoid and pleomorphic) derived from central, peripheral and metastatic sites. The intensity of c-Jun staining (from 0 (no staining) to 3+ (strong staining)) was scored independently by 2 pathologists.
Results: The majority of DDLPS exhibited moderate to strong c-Jun staining (average intensity = 2+; n=23). In contrast, c-Jun levels in pure WDLPS were low (average intensity = 0.6+; n=5). In those cases of DDLPS with a WD component, average c-Jun levels were lower in the WD component than the DD component (average intensities = 1.8+ for DD and 1.3+ for WD components; n=18; $p<0.03$), but higher than those seen in pure WDLPS ($p<0.03$). We also found that c-Jun levels were low in all cases of cytogenetically confirmed myxoid (average intensity= 0.4+; n=5) and pleomorphic (average intensity= 1+; n=2) liposarcomas.
Conclusions: We find that c-Jun, an inhibitor of adipocytic differentiation, is generally expressed at higher levels in DDLPS than WDLPS. However, the WD components of DDLPS often express higher c-Jun levels than pure WDLPS. We hypothesize that WD components with moderate c-Jun protein expression may represent a biologically intermediate state between DDLPS and pure WDLPS and that c-Jun immunopositivity in WDLPS may potentially be a harbinger of concurrent or incipient dedifferentiation. Additional studies are underway to determine the genetic basis of differential c-Jun expression in these tumors, as well as its expression in other sarcoma types.

70 Fluorescence In-Situ Hybridization (FISH) Is a Useful Ancillary Diagnostic Tool for Extraskeletal Myxoid Chondrosarcoma

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Background: Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma of uncertain differentiation. EMCs are typically characterized by a nodular growth pattern with reticular strands of eosinophilic cells with abundant myxoid stroma and can cause confusion with other myxoid sarcomas. Immunohistochemistry is usually non-specific. The majority of EMCs harbor a balanced t(9;22)(q22;q12) that fuses *EWSR1*with *NR4A3* (aka *CHN*). Other less common variant translocations involving *NR4A3* have also been described. We examined the diagnostic utility of FISH using an *EWSR1* break-apart DNA probe on formalin-fixed paraffin-embedded (FFPE) tissue for EMC.
Design: Eleven cases of EMC with FFPE tissue available were retrieved from the pathology files of our institution from 1990-2007 and clinical information obtained with prior IRB approval. Unstained coated slides were prepared and FISH was performed using the LSI *EWSR1* break-apart probe set (Vysis, Downers Grove, IL).
Results: The median age at presentation was 54 (30-73) years. There were 9 males and 2 females. All 11 cases were either consistent with or highly suggestive of the diagnosis though one case exhibited higher grade features. All eleven tumors occurred in the thigh, inguinal or gluteal region. Ten cases were analyzable by FISH of which nine (including the higher grade case) were positive for rearrangement of the *EWSR1* locus.
Conclusions: Nine of the ten analyzable EMCs contained a rearrangement at the *EWSR1* locus (22q12) detected by break-apart FISH probes. This underscores the prevalence of *EWSR1* rearrangements over the other described alternative translocations. FISH is effective in the diagnosis of EMC in most cases, and can differentiate it from other myxoid sarcomas lacking this rearrangement.

71 Identification of the ASPL/TFE3 Fusion Transcript and Immunohistochemical Detection of TFE3 in Formalin-Fixed Paraffin-Embedded Tissue: Their Role in the Diagnosis of Alveolar Soft Part Sarcoma (ASPS)

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Background: ASPS is a rare mesenchymal malignancy. Its diagnosis can be problematic due to histological overlap with other tumours as well as a lack of specific diagnostic markers. ASPS has a recently described unbalanced translocation, der(17)t(X;17)(p11;q25), with type 1 and 2 variants involving fusion of the first seven exons of ASPL to exon 6 (type 1) or 5 (type 2) of TFE3 (GenBank NM_006521). Anti-TFE3, a commercial antibody, recognizes the carboxy terminal portion of TFE3, resulting in strong nuclear staining.
Design: RNA was extracted from 13 formalin-fixed, paraffin-embedded cases of typical ASPS. Novel primers were designed to detect type 1 and type 2 fusions to produce PCR products of 120bp and 130bp, respectively. RT-PCR for both fusion transcripts was performed on 13 ASPS as well as 20 negative controls, including other sarcomas, metastatic carcinoma, melanoma and granular cell tumour. All PCR products were confirmed by DNA sequencing. Immunohistochemistry was carried out on 4μm paraffin sections of all samples using an anti-TFE3 goat polyclonal antibody (Santa Cruz Biotechnology) with the avidin-biotin peroxidase technique.
Results: RNA was successfully extracted from all 13 ASPS and 20 controls. All 13 ASPS contained a fusion transcript; 8 were Type 1 and 5 were Type 2. All 13 ASPS had strong nuclear immunostaining in at least 50% of the tumour cells. Four granular cell tumours, including a malignant one, showed variable degrees of nuclear staining; all of these were negative for the fusion transcript. All other tumours tested were negative with anti-TFE3.
Conclusions: 1) Anti-TFE3 is a sensitive marker, but not entirely specific for some of the simulators of ASPS. 2) RT-PCR techniques, designed for application on paraffin-embedded material, are highly sensitive and specific in detecting both ASPL/TFE3 fusion transcripts. 3) RT-PCR verification of immunopositive cases that are not entirely characteristic of ASPS histologically is recommended.

Breast

72 D2-40: An Additional Marker for Myoepithelial Cells of Breast

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Background: D2-40 is a recently available mouse monoclonal antibody specifically recognized human podoplanin and has been used in identifying lymphovascular invasion of tumors. Although its expression has been evaluated in other tissues, its use as a marker of the myoepithelial cells of breast has not been reported. We have found that it serendipitously stains the myoepithelial cells of the terminal duct lobular units, and its utility in breast pathology is therefore further explored.
Design: Histopathological materials of 48 patients with a variety of breast diseases were reviewed and paraffin embedded tissue blocks were chosen to include usual ductal hyperplasia (41 cases), atypical ductal hyperplasia (5 cases) and ductal carcinoma in situ (DCIS, 17 cases) for this study. Normal breast parenchyma and invasive carcinoma were also noted in some of the tissue sections. Immunohistochemistry for D2-40, p63 and Calponin was performed and the results were compared.
Results: D2-40 immunohistochemistry stains the cytoplasm of the myoepithelial cells along the periphery of benign proliferative lesions, atypical ductal hyperplasia and majority of DCIS, and in the luminal lesions of usual ductal hyperplasia. The staining pattern is identical to that of Calponin with less intensity. In addition the staining of

myofibroblasts is less and the background staining is lighter, therefore easier to interpret. Among the three markers, the p63 appears to the most sensitive and specific one. Due to the presence of non-specific stain of D2-40, precaution should be excised in interpreting retraction artifact when it is used as the lymphovascular marker, particularly when the glands are surrounded by rich myofibroblasts. The myoepithelial cells in normal breast parenchyma are constantly stained by all three markers, and they are absent in all the invasive carcinomas.

Conclusions: D2-40 immunohistochemistry reliably identifies the myoepithelial cells of the breast in a variety of lesions in a fashion similar to p63 and Calponin, and can be used as an additional marker.

73 Nuclear Grade and Survival in Invasive Lobular Carcinoma: A Study with Long-Term Follow-Up

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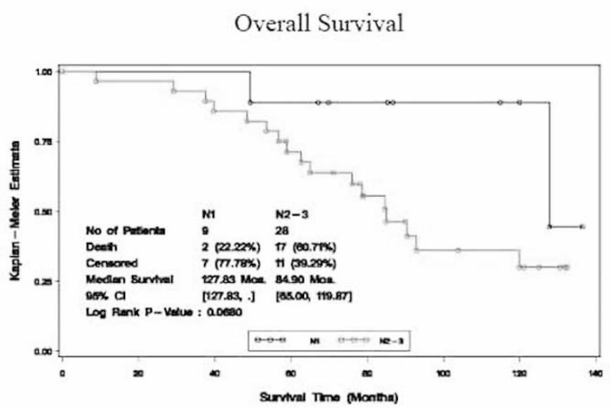
Background: Histologic grade is an important prognostic marker for invasive ductal carcinoma; however, its utility is less clear for invasive lobular carcinoma (ILC), given that 2 of the 3 criteria, tubule formation and mitoses, show little variation in ILC. Thus, with much of the emphasis placed upon the final criterion, nuclear pleomorphism, the objective of the current study is to determine the impact of nuclear grade of ILC on survival in patients with long-term follow-up.

Design: A consecutive series of histologically confirmed cases of ILC diagnosed at a single institution from 1992 to 1998 were reviewed and assigned a low (N1), intermediate (N2), or high (N3) nuclear grade based upon the degree of nuclear pleomorphism. The overall survival and relapse-free survival stratified by nuclear grade were compared using the Kaplan-Meier method.

Results: Thirty-seven patients were identified, 30 (81%) Caucasian and 7 (19%) African-American, with an average age of 60 years at diagnosis (range 36-79) and an average tumor size of 34 mm (range 5-120). Eighteen (49%) had lymph node metastasis. Nine patients (23%) had N1 grade tumors, 15 (41%) N2, and 13 (35%) N3. Clinical follow-up ranged from 9-136 months (median 79). Survival curves showed a trend for better overall and relapse-free survival for patients with N1 tumors compared to N2 or N3. No significant difference was present in overall or relapse-free survival for patients with N2 or N3 tumors. Given that survival curves for N2 and N3 tumors closely paralleled, overall and relapse-free survival were evaluated using a two-tiered system, N1 and N2-N3. A definite trend for improved overall survival was observed in patients with N1 tumors (p=0.07) (Figure 1). A similar trend was present with regard to relapse-free survival (p=0.06).



Conclusions: A two-tiered nuclear grading system appears to provide the same prognostic information as a three-tiered system and, given the inherent potential for interobserver variability, may be easier to implement. A multivariate analysis is ongoing and may further clarify the prognostic significance of nuclear grade in ILC.

74 Reduction Mammoplasty (RMP) Specimens for Macromastia from Women 40 Years and Older Should Undergo More Thorough Histological Evaluation Than Younger Women. A Prospective Study

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Background: RMP for macromastia or asymmetry is performed in an estimated 105 000 US women annually. Incidence of occult cancer in RMP ranges from 0.06% to 4.6%. No standard pathology assessment for RMP exists.

Design: This data represent 20 months of an ongoing prospective study. Gross pathologic evaluation and systematic tissue sampling were performed on all cases: 3 breast tissue sections and 1 skin section (tissue section set 1; TSS-1) were submitted for microscopy by a Pathology Assistant (PA). This is our routine RMP tissue sampling. For this study, additional breast tissue samples consisting of 4 sections in each set (TSS-2, TSS-3) were submitted by a second PA. All patients with significant pathologic findings (SPF) (carcinoma and atypical hyperplasia) had negative preoperative mammograms.

Results: A total of 202 cases were evaluated. SPF were present in 12.4% (25/2002; 95% CI: 8.2 to 17.7%) of patients (Table).

Significant findings according to tissue Section Set (TSS)

Diagnosis (n)	TSS-1[a]	TSS-2[b]	TSS-3[c]
ALH (14)	5	5	4
LCIS (3)	2	1	1
ADH (3)	1	1	1
DCIS (3)	2	1	-
Invasive (2)	-	2	-
Total (25)	10 (40%)	10 (40%)	5 (20%)

[a] Diagnosis made on TSS-1 regardless of findings on TSS-2 and/or TSS-3. [b] Diagnosis made on TSS-2, regardless of findings on TSS-3. [c] Diagnosis made solely on TSS-3.

64.4% (130/202) patients were 40 years and older. Age was significantly different in patients with and without SPF; (mean age: 51.2 vs. 42.9; t-test p=.002). 23/25 patients with SPF were 40 years and older (Fishers exact test, p= .002). Carcinoma was identified in 4% of all cases (2 invasive, 3 DCIS, 1 PLCIS and 2 LCIS; 95% CI: 1.7 to 7.7%); the rate of carcinoma was 6.2.% (8/130; 95% CI: 2.7 to 11.8%) in patients 40 years and older and 7.9% (6/76; 95% CI: 2.9 to 16.4%) in patients 50 years and older. 6 out of 8 carcinomas (75%) were in patients 50 years and older. Eight benign skin lesions were evident clinically and on gross examination.

Conclusions: A significantly higher rate (12.4%) of SPF was identified in our prospective RMP study than in previously published retrospective studies. Age was a significant factor for SPF. Almost all (23/25) significant findings were present in patients 40 years or older, supporting increased sampling in this group. Skin sections are unnecessary in the absence of apparent lesions.

75 Analysis of Chromosomal Imbalance Patterns in ERBB2 Positive Breast Cancer Using Comparative Genomic Hybridization and Correlation with Chromogenic In Situ Hybridization for Genes ERBB2, C-MYC, TOP2A, and CCND1

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Background: Comparative genomic hybridization (CGH) enables chromosomal imbalances in the entire tumor genome to be examined, detecting gains and losses in genetic material. We aimed to use CGH to determine chromosomal imbalances in a group of ERBB2+ breast cancers and to analyze the concordance between the results obtained by CGH and chromogenic in situ hybridization (CISH) in the study of the genes ERBB2, C-MYC, TOP2A and CCND1.

Design: CGH performed on frozen material from 10 cases of breast cancer with ERBB2 overexpression, according to the protocol previously reported. CISH was performed on 2-3µm-thick archived formalin-fixed paraffin-embedded tumor samples, using the probes ERBB2, C-MYC, TOP2A, CCND1.

Results: CGH found gains in 17q (90%), 8q (80%), 1q (80%), 16p (60%) and 20q (60%) and losses in 3p (50%), 5q (50%), 11q (50%), 14q (50%) and 13q (40%), with a mean of 20 alterations per case. High level amplifications were detected in 9 different chromosome regions, all previously reported. The profiles of gains obtained by CGH at 17q were variable: peaks of gain in 17q12-21.1, peaks of gain in both 17q11.2-q21 and another distal/terminal region, or gain in the entire 17q arm. The concordance between CGH results (gains in 17q12-qter) and CISH results (>6 signals/nucleus) for ERBB2 was 90% (9/10 cases). For the other genes, the correlation between the CISH and CGH results was: TOP2A (17q21.2) 70%, C-MYC (8q24) 88%, and CCND1 (11q13) 80%.

Conclusions: In conclusion, the group of ERBB2+ breast cancers had a high number of CGH-detected chromosomal alterations, characteristic of highly aggressive tumors and some related with a worse prognosis. CGH determinations of the profile of chromosomal alterations show high concordance with the CISH results for the specific genes studied.

76 Expression of *HER-2/neu* Splice Variants in the Human Breast Cancer Cells

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Background: In human HER-2/neu-positive advanced breast cancer, trastuzumab are widely applied options in the first-line setting. Protein overexpression and gene amplification are examined for the evaluation of HER-2/neu. However, many cases in which the protein expression does not correlate with the gene amplification have been reported. In addition, there are reports that the expression level of mRNA does not correlate with protein expression level of HER-2/neu. Recently, many splice variants of which the function is unknown reported for *HER-2/neu*. The objective of our study was to investigate the relation between expression pattern of splice variants and protein expression was analyzed for *HER-2/neu* in the human tumor cells.

Design: We detected the expression of eight *HER-2/neu* splice variants which listed on the NCBI (isoform a: NM_004448, isoform b: NM_001005862) or EMBL (ENST00000302390, ENSG00000141736 SP2 3, 6, and 9) in the human tumor cell lines (SK-BR-3, MDA-MB-453, CRL1500, HMC-1-8, MCF-7, and HeLa) by the RT-PCR. To prepare cDNA, total RNA extracted from tumor cells was primed by oligo dT primer or random hexamer. Protein expression in each tumor cell was confirmed in immunohistochemistry and western blotting.

Results: SK-BR-3, MDA-MB-453 and CRL1500 over-expressed HER-2/neu protein, while the expression of Her-2/neu protein was negative or low in the HMC-1-8, MCF-7 and HeLa cells. In the RNA expression, it became clear that, many variants were transcripted simultaneously in the tumor cells. In the oligo dT priming, the expression of the isoform a which encodes the protein correlated for the protein expression, while the isoform b was detected in Her-2/neu-positive or negative cells. In the random priming, all variants were detected in SK-BR-3 cells which express strongly the HER-

2/neu protein. However, the clear relation could not be recognized between protein expression and *HER-2/neu* variant expression except for the isoform a. In addition, it is interesting that *HER-2/neu* SP6 and SP8 that to be non-coding RNA, is detected even in the dT priming.
Conclusions: The expression of splice variants except for *HER-2 /neu* isoform a did not correlate with protein expression in human tumor cells. It is suggested that the disparity between RNA expression and protein expression for *HER-2/neu* may result from the simultaneous detection of multiple splice variants.

77 Breast Carcinoma with Luminal Phenotype: Prognostic Significance of Pathological and Immunohistochemical Findings

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Background: Luminal immunophenotype of breast carcinoma (BC) is defined by the positivity for estradiol and progesterone receptors (ER and PgR) and negativity for Her2. The aim of our study was to determine pathologic features and to assess the prognostic significance of the level of ER and PgR expression, the proliferative activity (Ki67), p53 and bcl-2 in a series of luminal subtype BC.
Design: A total of 747 cases of invasive BC were retrieved from the Surgical Pathology files. Median clinical follow-up was 74 months (range 6 to 245 months). Histologic grade (HG) was determined according to the modification of Elston-Ellis. Immunohistochemical (IHC) staining was performed for ER and PgR (10-49%=low level and ≥50%=high level), Her2 (2+ and <30% 3+ confirmed by FISH), Ki67 (cut-off 20%), p53 (cut-off 20%) and bcl2 (cut-off 50%). Significant associations were identified using Chi-square and Fisher´s exact test. Actuarial survival was calculated by the Kaplan-Meier method (log rank test). Multivariate analysis was determined by Cox´s proportional hazard model. A p-value <0.05 was considered significant.
Results: Based on IHC findings, 452 (60.5%) tumors were luminal subtype (ER/PgR ≥10%, Her2-negative). Tumors were predominantly of ductal type (87%), ≤20 mm in size (56.2%) and negative lymph nodes (66.4%). HG was G1 in 114 (28%) cases, G2 in 187 (41%) and G3 in 141 (31%). Increased Ki67 was observed in 25% of tumors, p53 was positive in 11% and low bcl-2 in 27%. High HG correlated with low level of ER and PgR, high Ki67, p53 positivity and low bcl2 (all p<0.000). Poor survival was seen for patients with larger tumors, of G3, positive lymph nodes, high expression of Ki67 and p53, and low bcl-2 and ER (≤50%) (all p<0.02). However, the level of PgR showed only a trend (p=0.073). Stratification of cases combining the Ki67 and p53 results showed poorer survival for the subset of patients with "high risk" (Ki67/p53 ≥20%) compared to those with "low risk" (Ki67/p53 <20%) (82% vs 94%; p<0.000). Multivariate analysis revealed that lymph node status, HG and Ki67/p53 were significant independent predictors of survival (all p<0.02).
Conclusions: Our findings in a series of BC of luminal subtype confirm that HG is a powerful prognostic factor independently of the lymph node status. Furthermore, stratification of BC of luminal subtype combining Ki67 and p53 data is more useful to define groups of risk than the levels of ER and/or PgR.

78 Comparison of ER and PR Assessment by Local IHC, Central IHC, and Central Quantitative RT-PCR in ECOG Breast Cancer Study 2197

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Background: Accurate laboratory assessment of hormone receptors (HR) in breast carcinoma is therapeutically important. We compared ER and PR measured by local laboratories using IHC and by central laboratories using IHC and quantitative RT-PCR by Oncotype DX.
Design: Tumors from 776 pts (179 of whom relapsed) enrolled on E2197 were examined; pts had 0-3 positive nodes and received doxorubicin and cyclophosphamide or docetaxel plus hormonal therapy (if HR+). Central IHC for ER (1D5) and PR (636) used two 1.0 mm tissue microarrays. The Allred score (AS) was used for ER/PR semiquantitation (AS>2 = pos). Quantitative RT-PCR analysis used Oncotype DX and pre-defined cutoffs of 6.5 and 5.5 units. Hormone receptor (HR) positive was defined as ER and/or PR positive. Local assessment used local protocols and cutoffs.
Results: Results from local IHC (776 pts) were compared with central IHC (769 pts) and RT-PCR (776 pts). The concordance between local or central IHC and central RT-PCR was high, and similar to the high concordance between local and central lab IHC (table). 6% of HR+ pts by central IHC (27/459) and 7% of local IHC (32/455) were HR- by RT-PCR. 8% of HR- pts by central IHC (24/302) and 12% of local IHC (38/321) were HR+ by RT-PCR. The likelihood of recurrence was significantly lower in HR+ than HR- pts by all three assays (p < 0.01). 1% of ER+ pts by central (5/409) and 5% of local (21/435) IHC were ER- by RT-PCR. 14% of ER- pts by central (50/360) and 13% of local (45/341) IHC were ER+ by RT-PCR. In ER+ pts receiving hormone therapy, Recurrence Score was a highly significant predictor of recurrence (p<0.001), whereas ER by central RT-PCR (p=0.05) and ER by central IHC Allred score (p=0.12) were not.
Conclusions: There is a high degree of overall concordance among local IHC, central IHC and central RT-PCR for ER, PR, and HR status. RT-PCR is an alternative for HR assessment.

% Concordance (95% CI)

Comparison	ER	PR	HR
Local IHC vs Central IHC	90 (88, 92)	84 (82, 87)	90 (88, 92)
Local IHC vs RT-PCR	91 (89, 93)	88 (85, 90)	91 (89, 93)
Central IHC vs RT-PCR	93 (91, 95)	90 (88, 92)	93 (91, 95)

79 Prognostic Significance of Basal and Luminal Markers in Triple-Negative Breast Cancer

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Background: Recently, many efforts had been focused on classification of breast cancers according to molecular features, with particular emphasis on triple-negative (TN) (ER-, PR- and HER2-) breast cancers. In this study, we examined the ability of immunohistochemical markers to predict survival in a large series TN breast cancer.
Design: We identified 147 TN breast cancers among 625 consecutive invasive breast cancers and compared clinicopathologic characteristics and patients' survival between TN and non-TN breast cancers. The TN cancers were further subclassified by staining for CK 5/6, EGFR, vimentin, c-Kit, p63, P-cadherin, CK8 and CK18. We then applied 4 different criteria to define basal-like phenotype (BLP) in TN breast cancers (criteria 1: CK5/6+ only, criteria 2: CK5/6+ and/or EGFR+, criteria 3: CK5/6+ and/or EGFR+ and/or vimentin+, and criteria 4: one or more marker(s) positive among CK5/6, EGFR, vimentin, c-Kit, p63 and P-cadherin). Each of these criteria, as well as each individual marker, was then evaluated for prognostic significance by survival analysis.
Results: TN breast cancers were found in 23.5% of invasive breast cancers. Compared with non-TN breast cancers, TN cancers showed larger tumor size and higher histologic grade, but fewer lymph node metastasis. In addition, patients with TN breast cancer had reduced overall survival (OS) within 6 years of diagnosis but not thereafter. On immunohistochemical stains, we noted CK5/6+ in 35.4%, EGFR+ in 16.3%, vimentin+ in 28.6%, c-Kit+ in 11.6%, p63+ in 8.0% and P-cadherin+ in 43.8% of TN breast cancers. Using the criteria outlined above, we defined 52 (35.4%) cases as BLP by criteria 1, 65 (44.2%) by criteria 2, 82 (55.8%) by criteria 3 and 113 (76.9%) by criteria 4. Remarkably, BLP defined by any of these criteria did not show survival difference from non-BLP in TN breast cancers. Interestingly, however, luminal CKs, 8 and 18 were also commonly expressed in TN breast cancers (55.1% and 45.6%, respectively), and TN breast cancers expressing CK8 and/or CK18 showed reduced OS (p=0.002) and disease-free survival (p=0.011).
Conclusions: In our series, TN breast cancers showed poorer prognosis within 6 years of diagnosis than non-TN breast cancers. However, there was no survival difference between BLP and non-BLP as defined by immunohistochemical profiles of 6 basal markers in TN breast cancers. By contrast, expression of luminal CKs appears to identify a more aggressive subgroup of TN breast cancers.

80 Molecular and Phenotypic Heterogeneity of Triple-Negative Breast Cancers

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Background: Breast cancer has long been recognized to be a heterogeneous disease, with variable morphological and clinical features. In recent years, there have been efforts to classify breast cancers according to molecular features, with particular emphasis on breast cancers that are negative for expression of ER, PR, and HER2 oncogene (triple negative). We examined the histomorphology and immunophenotypic characteristics of triple negative (TN) breast cancers to determine whether these tumors can be further subclassified.
Design: We performed immunohistochemical stains for CK5/6, EGFR, vimentin, p63, c-Kit, P-cadherin, CK8 and CK18 by using tissue microarrays that represent 212 TN breast cancers from patients in Korea and the United States. We also reviewed histology of all TN cancers and photographed representative histology of each case to match cases according to recurrent histologic patterns. Finally, we compared histomorphology with immunoprofiles of these cancers.
Results: TN cancers expressed CK5/6, vimentin, EGFR, c-Kit, p63 and P-cadherin in 39.2%, 32.5%, 14.6%, 19.8%, 9.4% and 51.4% of cases, respectively. CK5/6, vimentin, EGFR, c-kit were almost never expressed in hormone receptor-positive cancers and rarely expressed in HER2-positive cancers. Luminal cytokeratins (CK8 and/or CK18) were commonly expressed in TN breast cancers (54.5% of cases), and their expression was independent of CK5/6 expression (r=-0.15). There were no significant correlations for expression among these six markers commonly used to define the "basal-like phenotype" (r=-0.017~0.163), and thus clustering analysis could not define subclasses within the set of TN breast cancers. Morphology of the TN cancers was also heterogeneous, although subsets of cancers with similar histologic appearance could be recognized. However, classifying TN cancers by morphological appearance did not correlate with any classification by immunophenotype.
Conclusions: TN breast cancers are very heterogeneous with respect to immunophenotype and morphology. A significant percentage of TN breast cancers have an immunophenotype suggestive of luminal cell lineage, and these cancers do not have a morphological appearance that is appreciably different than TN cancers with basal marker expression. Even among cancers that express basal markers, there is significant morphological and immunophenotypic heterogeneity. Thus, the classifier of basal-like phenotype of breast cancer does not define a distinct, homogeneous group of breast cancers.

81 A Kaiser-Permanente Population-Based Study of Breast Cancer ER and PR Expression by the Standard Method, Immunohistochemistry (IHC), Compared to a New Method, Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

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Background: Assessment of ER and PR is of significant importance in breast cancer diagnosis and treatment. We compared assessment of ER and PR by central lab IHC to central lab RT-PCR by Oncotype DX™.

Design: Breast cancer specimens from the Kaiser Oncotype DX study (Habel et al, Breast Can Res 2006) were evaluated by IHC for ER (SP1) (Cheung et al, JCO 2006) and PR (636) using ≥1% staining for positivity. Standardized quantitative RT-PCR analysis for ER and PR was performed using pre-defined cutoffs of 6.5 and 5.5 units, respectively, for positivity. Hormone receptor (HR) positivity was defined as ER and/or PR positive. Reference normalized expression measurements ranged from 0 to 15, where each 1-unit increase reflects about a 2-fold increase in RNA.
Results: Evaluable data was obtained in 607 pts (94% of the published cohort). A two-by-two table (below) compares IHC versus RT-PCR. The overall concordance (95% CI) between IHC and RT-PCR was 96% (94%-97%) for ER, 90% (87%-92%) for PR, and 95% (93%-97%) for HR (all p<0.0001). The kappa (95% CI) between IHC and RT-PCR was 83% (77%-89%) for ER, 76% (70%-82%) for PR, and 81% (75%-88%) for HR (all p<0.0001). Of the 20 discordant pairs that are ER negative by IHC but ER positive by RT-PCR, 17 (85%) are within 1 unit of the 6.5 cutoff. Of the 38 discordant pairs that are PR negative by IHC but PR positive by RT-PCR, 23 (61%) are within 1 unit of the 5.5 cutoff. Of the 7 discordant pairs that are ER positive by IHC but ER negative by RT-PCR, 6 (86%) are within 1 unit of the 6.5 cutoff. Of the 22 discordant pairs that are PR positive by IHC but PR negative by RT-PCR, 19 (86%) are within 1 unit of the 5.5 cutoff.
Conclusions: There is a high degree of overall concordance between central IHC and central RT-PCR for ER, PR and HR status. RT-PCR by Oncotype DX for ER, PR and HR status is an alternative to IHC.

	ER+ IHC	ER- IHC	PR+ IHC	PR- IHC	HR+ IHC	HR- IHC
RT-PCR+	501	20	403	38	502	22
RT-PCR-	7	79	22	144	7	76

82 Analysis of Revised Nottingham Tumor Grade Constitutive Components and Recurrence Free Interval in ECOG Breast Cancer Study E2197

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Background: Tumor grade has been shown to be a predictor of recurrence in invasive breast cancer. This study evaluates the relationship between tumor grade components and relapse-free interval in hormone receptor positive (HR+) and negative (HR-) treated patients from ECOG 2197.
Design: A sample of tumors from 776 patients (179 of whom relapsed) with sufficient tumor tissue enrolled in E2197 was examined; all patients had 0-3 positive nodes and received four 3-week cycles of doxorubicin (60 mg/m2) and cyclophosphamide 600 mg/m2 (AC) or docetaxel 60 mg/m2 (AT) plus hormonal therapy (if HR+). There was no difference in outcome for the two treatment arms after a median followup of 6.3 years. Tumor grade was assessed centrally using the Nottingham criteria (Elston CW, 1991) by a single pathologist. The components of central grade were evaluated: tubule formation, nuclear pleomorphism and the number of mitotic figures. Cox proportional hazards analysis using the weighted pseudo partial-likelihood method was used to evaluate the relationship between central tumor grade components and relapse-free interval in HR+ and HR- patients separately.
Results: Results from 776 patients were evaluated. In the HR+ subgroup, overall histologic grade was significantly associated with recurrence (p<0.0001). Higher mitotic figures (>20) compared to low mitotic figures (≤9) was significantly associated with recurrence (p<0.0001). Nuclear variation and tubule formation appeared to have little association with recurrence after adjusting for mitotic index. In the HR- subgroup, overall histologic grade was not significantly associated with recurrence (p=0.64). Higher mitotic figures (>20) compared to low mitotic figures (≤9) was significantly associated with recurrence (p=0.025). Nuclear variation was significantly associated with recurrence but in an unexpected direction (p=0.022) comparing the large nuclei group to the mild and moderate group combined. Tubule formation appeared to have little association with recurrence.
Conclusions: In breast cancer patients treated with chemotherapy and hormonal therapy (if HR+), mitotic figures (>20) compared to low mitotic figures (≤9) was significantly associated with recurrence in both the HR+ and HR- patients; differences between HR+ and HR- patients deserve further study.

83 Heterogeneity of Quantitative RT-PCR Measurement of Estrogen and Progresterone Receptor Expression: Comparison of Tissue Microarray Cores to Whole Sections of Paraffin Embedded Breast Cancer Tissue

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Background: Tumor heterogeneity has been described in semi-quantitative studies performed using immunohistochemistry. To evaluate hormone receptor expression heterogeneity using a standardized, quantitative RT-PCR assay, we conducted a pilot study to determine whether the Oncotype DX™ assay can be performed with limited 0.6 mm tissue microarray (TMA) cores, and compared ER and PR gene expression in cores and whole sections.
Design: Four single cores (0.6 mm diameter, ~0.2 mm long) and two 5 um Whole sections were taken from 8 unique patient tumor blocks prepared from excisional breast cancer samples from the IMPACT trial. RNA was extracted and total RNA content was determined using RiboGreen fluorescence. The standard Oncotype DX 21 gene assay was performed on 375 ng RNA. Reference normalized expression measurements ranged from 0 to 15, where each 1-unit increase reflects about a 2-fold increase in RNA. Descriptive statistics (mean and SD) were used to compare ER and PR expression between the cores and the whole sections and analysis of variance was used to assess variability.
Results: Sufficient total RNA (>375 ng) was obtained in all 32 TMA cores. Gene expression profiles had strong signals and met criteria for successful RT-PCR. Intra-patient ER and PR results were similar in the two whole sections (SD = 0.40 and 0.25 units, respectively). Intra-patient core ER and PR results were similar to each other and to whole section values, although the intra-patient variability of the cores for ER and PR (SD = 0.90 and 0.90, respectively) were greater than the variability of the whole sections. In one case, variability of the cores could be attributed to sampling of tumor-poor regions.
Conclusions: Quantitative expression of ER and PR using Oncotype DX is very similar between replicates of whole sections and TMA cores taken from the same block.

84 Lobular Involution and Subsequent Breast Cancer Risk: Findings from the Nurses' Health Studies

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Background: Lobular involution is a physiologic process that occurs in the breast at varying rates as a woman ages. It has been hypothesized that lobular involution is inversely associated with subsequent breast cancer risk, but few prior studies have addressed this issue.
Design: We examined the relationship between lobular involution and the risk of subsequent breast cancer risk in a case-control study of benign breast disease (BBD) and breast cancer risk nested within the Nurses' Health Studies. Cases were women with breast cancer who had a prior benign breast biopsy (n=200). Controls also had a prior benign breast biopsy but were free from breast cancer at the time the corresponding case was diagnosed (n=915). Benign breast biopsy slides were reviewed by pathologists blinded to case/control status and were categorized as showing either nonproliferative changes (NP), proliferative disease without atypia (PWA), or atypical hyperplasia (AH). Normal terminal duct lobular units were categorized as showing complete involution, partial involution or no involution. Logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (CI) for the association between lobular involution and breast cancer risk, adjusting for age, year of benign biopsy, years of follow-up, family history of breast cancer, menopausal status and category of BBD.
Results: When compared with women whose benign breast biopsies showed no or partial involution, those whose biopsies showed complete involution (54 cases; 321 controls) had a 29% decrease in the risk of breast cancer (adjusted OR = 0.71, 95% CI = 0.49-1.03). Moreover, complete lobular involution was associated with a reduction in breast cancer risk for women in each of the three BBD categories when compared with those whose biopsies showed no or partial involution (NP: OR=0.68, 95% CI=0.32-1.42; PWA: OR=0.82, 95% CI=0.49-1.40; AH: OR=0.61, 95% CI=0.28-1.36).
Conclusions: The results of this study indicate that complete lobular involution in a benign breast biopsy is associated with a 29% reduction in breast cancer risk, even when adjusted for category of BBD. This suggests that lobular involution may be an important marker of decreased breast cancer risk in women with benign breast biopsies.

85 Basal-Like Invasive Breast Carcinoma: How Specific Are Immunohistochemical Markers?

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Background: The basal-like variant of invasive breast carcinoma was originally defined by gene expression profiling and is associated with a poor prognosis. By immunohistochemistry (IHC), these carcinomas lack estrogen receptor (ER), progesterone receptor (PR), and HER2 (so-called "triple negatives"), but they have been demonstrated to express markers such as cytokeratin 5/6, p63, c-kit, epidermal growth factor receptor (EGFR), and p53. While previous studies have shown the sensitivities of these latter markers to range from 50-76%, there have been no systematic IHC studies of their specificity. To further define the utility of these markers in the diagnosis of basal-like breast carcinoma, a series of non-basal breast carcinomas were studied to determine specificity.
Design: Biopsy and resection specimens from 138 non-basal-like infiltrating carcinomas (defined as positive for ER and/or PR and/or HER2) were analyzed by IHC for expression of the basal-associated markers cytokeratin 5/6, p63, cKit, EGFR, and p53, using standard methods and commercially available antibody clones. Results were compared with sensitivities of these markers in basal-like carcinomas as previously published by this laboratory.
Results: Results are tabulated below, along with reported sensitivities of the markers from a previous study that included 124 basal-like breast carcinoma cases.

Basal-like Marker Specificity

	p53	p63	CK 5/6	EGFR	cKit
Positive in non-basal-like	17/138 (12%)	12/138 (9%)	7/138 (5%)	21/138 (15%)	25/138 (18%)
% Positive in basal-like*	56%	50%	76%	70%	65%
Specificity	88%	91%	95%	85%	82%

* data from Goldstein LC et al, Lab Invest 81(Suppl. 1):32A, 2007

Conclusions: Markers associated with the basal-like phenotype of invasive breast carcinoma (cytokeratin 5/6, p63, c-kit, EGFR, and p53) are found in relatively few non-basal breast carcinomas, indicating specificities ranging from 82-95%. Cytokeratin 5/6 expression appears to be the single best marker of basal-like carcinoma, with specificity of 95% and reported sensitivity of 76%.

86 CK8/18 Expression Profiles and the Diagnostic Utility of a CK5/p63/CK8/18 Antibody Cocktail in the Evaluation of Papillary Lesions of the Breast

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Background: The distinction between breast papillomas (PAPs) and papillary carcinomas (PCs) is usually made histologically; however, immunohistochemistry for basal cytokeratins and p63 can also aid in this distinction. On the other hand, expression

of luminal cytokeratins (CK8/18) has not been well-characterized in papillary lesions of the breast. The aim of this study was to analyze such expression, as assessed by a CK5/p63/CK8/18 antibody cocktail, and to determine the diagnostic utility, if any, of using such a cocktail in the evaluation of papillary lesions of the breast.
Design: Breast papillary lesions were stained with a p63/CK5/CK8/18 antibody cocktail and interpreted in a blinded fashion. Depending on the number of cells with nuclear staining within the lesion (not the periphery), p63 reactivity was scored as negative, focal, moderate or diffuse. CK5 and CK8/18 were evaluated semiquantitatively by multiplying the intensity of staining (0, 1, 2, and 3+) by the percentage of cells stained at each intensity level and adding the results to arrive at a final intensity score (IS) (ranging from 0-300) for each marker.
Results: Thirty nine cases (22 PAPs and 17 PCs) have been evaluated to date. Diffuse p63 expression was seen in 21 PAPs (95%) but in none of the PCs ($P < 0.001$), which were negative [9 cases (53%)], or had focal [6 cases (35%)] to moderate expression [2 cases (12%)]. CK5 was expressed in both PAPs and PCs (100% vs. 71% of cases; $P = 0.01$), but at different levels as mean ISs were 114 and 28, respectively ($P = 0.001$), and strong expression (in ≥10% of cells) was limited to PAPs (36% of cases vs. 0% of PCs; $P < 0.001$). CK8/18 was expressed in all PAPs and PCs, also at different levels as mean ISs were 144 and 231, respectively ($P = 0.003$), and strong expression (in ≥10% of cells) was seen in all PCs vs. 55% of PAPs ($P = 0.002$). Additionally, the mean proportion of CK8/18-positive cells in PCs was higher than that in PAPs (94% vs. 34%; $P < 0.001$), with expression in >95% of cells seen only in PCs (76% of cases vs. 0% of PAPs; $P < 0.001$).
Conclusions: Although CK8/18 is expressed in both breast PAPs and PCs there is greater and stronger expression in PCs, which, along with differences in p63 and CK5 expression profiles, as detected by a CK5/p63/CK8/18 antibody cocktail, can help distinguish between these lesions. Evaluation of more cases, including atypical papillomas, and additional analysis is being performed to further determine the diagnostic utility of this antibody cocktail.

87 Molecular Profiling of *BRCA1* and *BRCA2*-Associated Breast Cancers Identifies FGFR2 as a Gene Differentially Expressed in *BRCA2*-Associated Breast Cancers

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Background: *BRCA1*- and *BRCA2*-associated tumors have many morphologic characteristics in common, but appear to have distinct molecular signatures. *BRCA1*-associated tumors are predominantly basal-like cancers, whereas *BRCA2*-associated tumors have a predominant luminal-like phenotype. To elucidate novel genes involved in these two spectra of breast cancer tumorigenesis we performed global gene expression analysis on breast tumors from germline *BRCA1* and *BRCA2* mutation carriers.
Design: Breast tumor RNAs from 7 germline *BRCA1* and 6 germline *BRCA2* carriers were profiled using UHN human 19K cDNA microarrays. Supervised univariate analyses were conducted to identify genes differentially expressed between *BRCA1* and *BRCA2*-associated tumors. Discriminatory genes were validated using real time reverse transcriptase polymerase chain reaction (RT-PCR) and/or immunohistochemistry or in situ hybridization on tissue microarrays containing an independent set of 58 *BRCA1* and 64 *BRCA2*-associated tumors.
Results: Genes more highly expressed in BRCA1-associated tumors included stathmin/oncoprotein 18, osteopontin, TGFß2 and Jagged 1 in addition to genes previously identified as characteristic of basal-like breast cancers. *BRCA2*-associated cancers were characterized by the higher relative expression of amongst others, *FGF1* and *FGFR2*. Tissue microarrays were used to validate the expression of FGFR2 protein by immunohistochemistry and Jagged 1 expression by *in situ* hybridization. *BRCA2*-associated cancers expressed higher levels of FGFR2 protein than *BRCA1*-associated cancers ($p=0.004$); whereas *BRCA1*-associated tumors exhibit elevated levels of Jagged1 mRNA compared to *BRCA2*-associated cancers ($p=0.02$).
Conclusions: FGFR2 and FGF1 were more highly expressed in *BRCA2*-associated cancers as compared to *BRCA1*-associated breast cancers, suggesting the existence of an autocrine or paracrine stimulatory loop. In addition to re-affirming the basal-like signature of *BRCA1*-associated tumors, we identified osteopontin, stathmin/oncoprotein 18, TGFβ2, and Jagged 1 as being overexpressed in *BRCA1*-associated tumors.

88 Mucinous Variant of Micropapillary Breast Carcinoma: An Aggressive Neoplasm That Needs To Be Distinguished from a Low-Grade Mucinous (Colloid) Carcinoma

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Background: Invasive micropapillary carcinomas are aggressive tumors known to have a propensity for early lymphatic invasion and a high frequency of lymph node metastases and local recurrence. Recognition of micropapillary carcinomas is important to ensure proper patient management. We have encountered an unusual (or under-recognized) pure mucinous variant of micropapillary breast carcinoma, which may mimic a low-grade mucinous carcinoma and should be distinguished from the latter to avoid under-treatment.
Design: We identified 14 cases of invasive micropapillary breast carcinoma exhibiting pure mucinous pattern, and evaluated the clinicopathologic features and HER2 status of these tumors.
Results: The patients were all women, 36 to 80 years of age (mean = 59 yrs). The tumors ranged in size from 1.1 cm to 4.5 cm (mean = 2.1 cm). All tumors demonstrated large amounts of extracellular mucin, absence of non-mucinous components, and the presence of characteristic floret-like micropapillary clusters of tumor cells and psammoma-body type calcifications. Lymphovascular invasion was identified in 9/14 cases (64%). Axillary lymph node metastases at presentation were found in 5/14 cases (36%). In additional 3 cases, isolated tumor cells and/or clusters (< 0.2 mm) were identified in the sentinel lymph nodes. HER2 protein overexpression (2+ to 3+) characterized 3/14 tumors (21%) and was associated with HER2 gene amplification.
Conclusions: Awareness of the morphologic spectrum of micropapillary breast carcinoma is important to ensure proper patient management.

89 Human Breast Carcinosarcomas Exhibit a Biphasic Her-2/neu Expression Pattern Due to Alterations in Receptor Heterodimerization and Downstream Signaling

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Background: Human breast carcinosarcomas are an example of epithelial-mesenchymal transition (EMT), in which there is a progression from a carcinomatous gene expression profile to a sarcomatous one and a concomitant pathological change from epithelial to mesenchymal histology. The effects of this transition on Her-2/neu expression have not been previously studied.
Design: We decided to study 30 cases of human breast carcinosarcomas which we confirmed as such by E-cadherin, cytokeratin, β-catenin and vimentin biphasic immunoreactivity. We deliberately excluded cases of low grade metaplastic carcinomas and cases of squamous cell carcinomas with spindle cell areas from this study and focused on true carcinosarcomas. We studied Her-2/neu by FISH and IHC. Based on these initial observational studies, we induced EMT in a human breast carcinoma cell line, HTB20, by serum deprivation and TGF-β treatment and studied Her-2/neu expression by FISH, Northern / Western blot and IHC and receptor dimerization /signaling by additional studies.
Results: In 15 (50%) of the breast carcinosarcomas, Her-2/neu was amplified 15-50 fold by FISH. This amplification was homogeneous, being present equally in the carcinomatous areas as well as the sarcomatous areas. Her-2/neu expression by IHC however was clearly biphasic, being overexpressed (3+) in the carcinomatous areas but weak to absent (0, 1+) in the sarcomatous areas despite the persistence of marked gene amplification by FISH. The HTB20 human breast carcinoma line which manifested prominent Her-2/neu gene amplification (40 fold) by FISH underwent a dramatic switch from an epithelial to a mesenchymal phenotype by serum deprivation and TGF-β treatment. This switch did not alter Her-2/neu gene amplification, mRNA or protein expression but down regulated Her-2/neu immunoreactivity by IHC. Subsequent immunoprecipitation and Western blot studies showed that receptor heterodimerization and downstream signaling through the mitogen-activated protein kinase (MAPK) pathway was inhibited.
Conclusions: Human breast carcinosarcomas illustrate that their EMT alters Her-2/neu heterodimerization and downstream signaling despite the persistence of gene amplification. These findings indicate that breast carcinosarcomas, as they undergo EMT, would not present the target for trastuzumab (Herceptin) and hence would be expected to exhibit progressive resistance to this monoclonal antibody despite the fact that carcinosarcomas remain Her-2/neu FISH positive.

90 The CSF-1 Response Signature in Breast Carcinoma

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Background: We have previously shown that soft tissue tumors (STTs) can be used as discovery tools in a genome-wide search to discover groups of novel markers that identify distinct types of tumor stroma (West PLoS Biol, 2005; e187). In a separate study, we found that CSF-1 is over-expressed in tenosynovial giant cell tumor (TGCT) and pigmented villonodular synovitis (PVNS) due to a chromosomal translocation of the CSF-1 gene, which results in the recruitment of macrophages into the tumor (West PNAS, 2006; 103: 690). In the current study, we seek to evaluate whether the CSF-1 response signature as observed in TGCT and PVNS defines a distinct stromal reaction pattern in breast cancer.
Design: Significance analysis of microarrays was performed to define the CSF-1 response signature, which consists of 898 genes that showed significantly increased expression in PVNS (n=8) and TGCT (n=7) as compared with desmoid type fibromatosis (n=7) and solitary fibrous tumor (n=6), with a false discovery rate of 0.02%, based on gene expression profiling previously performed (West PNAS, 2006; 103: 690). The expression of genes and proteins in the CSF-1 response signature was evaluated in six breast cancer gene expression datasets (n=1066) and a breast cancer tissue microarray (TMA) (n=283).
Results: Unsupervised hierarchical clustering demonstrates that a subset of breast cancers (18 - 33%) show coordinated high levels of expression of a subset of genes from the CSF-1 response signature. Gene ontology annotation suggests that these genes encode proteins expressed in macrophages, lymphocytes, and stromal cells and involved primarily in the immune response. These breast cancers tend to be higher grade ($p<0.001$), ER negative ($p=0.001$), PR negative ($p<0.001$), exhibit p53 mutations ($p<0.001$), belong to the luminal B molecular subtype ($p=0.034$), and show a trend towards decreased overall survival ($p=0.067$). On a breast cancer TMA, a subset of tumors (11%) show coordinated expression of four proteins from the CSF-1 response signature (CD32, CSF1, CD16, and cathepsin L). These tumors tend to be higher grade ($p<0.001$), ER negative ($p<0.001$), and PR negative ($p=0.001$).
Conclusions: Our data show that an expression signature defined by STTs can be used to characterize a distinct stromal reaction pattern seen in breast carcinomas and associated with a particular clinicopathological phenotype. We believe that these studies will lead to the identification of novel prognostic markers and potential therapeutic targets in breast tumor stroma.

91 The Fibromatosis Stromal Signature in Breast Carcinoma

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Background: The tumor micro-environment is an important contributor to carcinogenesis; however, the gene and protein expression patterns that characterize tumor stroma remain poorly understood. We have previously used soft tissue tumors (STTs) as discovery tools in a genome-wide search to discover groups of novel markers that identify distinct types of tumor stroma. In our initial study, we demonstrated that evaluation of gene expression profiles of desmoid-type fibromatosis (DTF) and solitary fibrous tumor can be used to identify distinct stromal reaction patterns in a breast carcinoma dataset with prognostic implications (PLoS Biol 2005; e187).

Design: We now evaluate the expression of DTF genes in five additional breast cancer gene expression datasets with clinical outcome (n=771) and identify a core set of DTF genes that are consistently coordinately expressed in a subset of breast cancers (the DTF-B gene set). Using tissue microarrays (TMAs), we evaluate the expression of DTF-B proteins in the tumor microenvironment of breast carcinomas.

Results: Unsupervised hierarchical clustering demonstrates that a subset of breast cancers (23-35%) show coordinated high levels of expression of DTF-B genes. These breast cancer cases tend to be lower grade ($p<0.001$), express ER ($p=0.051$), belong to the luminal A ($p=0.031$) or normal ($p<0.001$) molecular subtypes, and are less likely to belong to the basal ($p=0.002$), or luminal B ($p<0.001$) subtypes. The breast cancers defined by the DTF-B gene signature show significantly increased recurrence free survival ($p = 0.001$), breast cancer free survival ($p = 0.049$), and overall survival ($p = 0.020$). Using a breast cancer TMA containing samples from 283 patients, we show that breast cancers defined by coordinate stromal expression of two DTF-B proteins (SPARC and CSPG2) are significantly less likely to have a lymph node metastasis at the time of diagnosis ($p=0.006$). Evaluation of SPARC on an additional breast cancer TMA containing cores from 434 cases (median follow-up of 15.4 years) shows that strong SPARC stromal staining shows a trend for increased breast cancer free survival ($p=0.106$).

Conclusions: Our data confirm that a gene set defined by a soft tissue tumor can be used to characterize a distinct gene and protein expression pattern in the micro-environment of breast carcinoma with prognostic implications.

92 CK5 Is More Sensitive Than CK5/6 in Identifying "Basal-Like" Carcinoma of the Breast

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Background: Multiple immunohistochemical (IHC) stains, including CK5/6 are used in a panel format to identify "basal-like" carcinomas. We set to determine the sensitivity and specificity of a new CK5 antibody and compared its expression to CK5/6 in a variety of breast carcinoma cases.

Design: The study was performed on 3 breast carcinoma tissue microarrays (TMAs). Each tumor was represented by 3 cores (3-fold redundancy). Each core diameter was 0.6mm. The TMA details are as follows: TMA 1 (n=59): Consecutive cases of 48 ductal carcinomas, 8 lobular (6 classic, and 2 pleomorphic), 2 mixed ductal and lobular, and 1 metaplastic carcinoma (mixed adenocarcinoma and chondrosarcoma components). TMA-2 (n=16): Basal-like breast carcinomas in women <40 years of age. TMA-3 (n=11): Basal-like breast carcinomas in women 40 years and older. The basal-like carcinomas represented on TMA-2 and TMA-3 have been previously characterized. They were all triple negative tumors with characteristic basal-like morphology. These tumors have been subjected to a panel of IHC stains known for identifying basal-like carcinomas (CK5/6, CK14, CK17 and EGFR). They all have shown some staining for at least 2 of the above 4 markers. Antibodies Used: CK5 (clone XM26, dilution 1:25, Novocastra) and CK5/6 (clones D5 and 16B4, predilute, Ventana). Scoring: H-score methodology was used to report scores for IHC stains in the current study. This method takes into account percentage as well as intensity of staining. The score ranges from 0-300. A score of 10 or less was considered as negative.

Results:

Comparison between CK5 and CK5/6 (all cases)

	CK5+	CK5-	Total
CK5/6+	20	0	20
CK5/6-	14	52	66
Total	34	52	86

Twenty cases positive for both CK5 and CK5/6 were either well characterized "basal-like" carcinomas from TMA 2 and 3 (n=15) or TMA 1 cases that showed characteristic basal-like morphology with triple negative status (n=5). For all positive cases, the percentage and intensity of staining was much higher with CK5 than with CK5/6. Of the 14 cases positive for CK5 and negative for CK5/6, 12 were "basal-like" carcinomas from TMA 2 and 3 and two cases from TMA 1. Although these 2 cases were morphologically "basal-like", they showed weak expression for either ER (H-score of 20) or PR (H-score of 90). There was no case that was negative for CK5 and positive for CK5/6.

Conclusions: Our results provide convincing evidence that CK5 is a more sensitive marker than CK5/6 in detecting "basal-like" breast carcinoma. There is no loss in specificity in transitioning from CK5/6 to CK5.

93 The Dynamic Biology of E-Cadherin and p120 Catenin: Development of a Dual Immunostain for Diagnostic Use in Breast Pathology

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Background: Morphologically, breast tumors are classified as either ductal or lobular in differentiation. ECAD and p120 have a dominant membrane pattern in ductal neoplasms, while lobular neoplasms lack ECAD but show strong upregulation of cytoplasmic p120. In approximately 5-10% of cases, including mixed ductal-lobular carcinomas, individual stains for ECAD and p120 may be difficult to interpret. Our goal was to develop a dual stain for ECAD-p120 for diagnostic use that discriminates ductal-lobular differentiation.

Design: Ten invasive ductal carcinomas (IDC), 5 invasive lobular carcinomas (ILC), and 7 in-situ lobular lesions were studied with double immunostain for ECAD (brown) and p120 (red) using the Ventana BenchMark® XT platform. The protocol consisted of a cell conditioning pretreatment followed by incubation of the 1st antibody, ECAD (clone ECH-6, Ventana) which was detected with Ventana's ultraView™ Universal DAB detection kit. p120 catenin (clone 98, diluted 1:200; BD Biosciences) was then applied followed by ultraView™ Universal AP Red detection kit. Staining intensity for both ECAD and p120 was graded on a scale of 0-4+.

Results: All IDC (4 with ductal carcinoma in situ) showed either 3+ or 4+ ECAD membranous staining. The color and intensity was somewhat variable in an individual tumor. All tumors showed part brown and red (magenta) membrane stain (i.e. both ECAD and p120). Normal ducts showed pale (pink) cytoplasmic staining for p120 while ductal neoplasms showed variable p120 that had an inverse relationship with the amount of membrane ECAD. All lobular lesions (invasive, in-situ carcinomas and atypical lobular hyperplasia) showed strong cytoplasmic red p120 staining and lacked ECAD. Pagetoid extension of neoplastic cells and early lobular neoplasia were starkly displayed.

Conclusions: (1) The double immunostain demonstrates the unique biological relationship between E-cadherin membrane intensity and p120 cytoplasmic expression intensity. The dual immunostain is very sensitive and even demonstrates the small cytoplasmic pool of p120 in normal ducts and ductal lesions. (2) Lobular neoplasia shows intense red cytoplasmic p120 catenin intensity due to upregulation as a result of complete ECAD loss. The dual stain is particularly useful in demonstrating small lobular lesions and pagetoid extension. (3) The dual stain will be helpful to observe the biology of these carcinomas and in diagnosing mixed ductal and lobular carcinomas.

94 *Ex Vivo* Predictive Biomarkers and Pharmacodynamic Assay in Breast Cancer

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Background: During recent years, a number of biomarker assays with predictive potential in cancer have been developed. The pharmacodynamic (PD) assays represent an essential tool to determine target population for individualized therapies in cancer patients and to define the optimal biological dose (OBD). By exposing cultured tumor cells to a cytotoxic agent is possible to determine whether a patient is likely to respond to a specific treatment or not. However, culturing cells from specific tumor types, as breast cancer, is rarely achieved, and, importantly, cultured cells *in vitro* do not mimic the complex tissue architecture and interactions of an *in vivo* primary tumor. To study the sensitivity of breast cancer to doxorrubicin, a standard treatment for advanced breast cancer, and predict the response of individual tumors to the drug, a human *ex vivo* tissue culture model has been developed based on fresh tumor tissues directly retrieved from patients.

Design: Surgical tumor samples from 31 patients with invasive breast carcinoma were excised, cut into 3 mm thick sections and cultured during 24 and 48 hours in F12 medium supplemented with 10% FBS in the presence or absence of 2 ug/ml doxorubicin and then, assayed by immunohistochemistry. DNA damage induced by the drug was demonstrated by p53 expression. Activation of signaling pathways ERK1/2 and AKT, and their regulators MKPs were investigated in order to measure doxorubicin activity on the tumors. Finally, downstream effects, including proliferation (Ki67) and apoptosis (TUNEL and cleaved caspase 3) were evaluated.

Results: Doxorubicin-DNA binding was demonstrated by fluorescence microscopy in cells and p53 expression was upregulated in all cultured treated tumors. Proliferation was reduced in 22 (71%) specimens and tumor apoptosis was achieved in 27 (87%) in a time-dependent manner. In addition, downregulation of ERK1/2 signaling was achieved in 9 (29%) specimens and AKT was inhibited in 12 (39%). These observed effects did not correlate with tumor grade, receptor status or HER2, but, interestingly, phosphatase MKP-1 expression seems to be related ($p<.001$) to the doxorubicin sensitivity.

Conclusions: This *ex vivo* tumor breast cancer model permits to explore the activation of molecular pathways and the presence of sensitivity biomarkers in their original microenvironment. This model might provide a useful tool to evaluate the efficacy of anti-cancer drugs, to define the OBD and to predict the response to targeted therapies.

95 Immunosuppressive Regulatory T Cells Are Associated with More Aggressive Breast Cancer Phenotypes: A Potential Therapeutic Target

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Background: FoxP3 is a marker for immunosuppressive CD25+CD4+ regulatory T cells (Treg). These Tregs are thought to play a role in inducing immune tolerance to antigens. There is increasing evidence that Tregs may be selectively recruited by carcinomas that are associated with a worse prognosis. We investigated whether breast carcinomas had significant numbers of FoxP3 positive Tregs by immunohistochemistry, and if their presence was associated with Nottingham grade (NG) or immunohistochemical profile.

Design: Ninety-seven needle core or excisional breast biopsies with invasive breast carcinoma diagnosed at the University of Washington were stained with antibodies to FoxP3, ER, and Her2. Nineteen cases were NG I, 26 cases were NG II, and 52 cases were NG III. The numbers of FoxP3 positive cells were counted manually in 3 high powered fields (40x) by two independent pathologists. The average scores were then correlated with the parameters of interest. A threshold of ≥ 15 FoxP3 positive cells/high powered field was used to define a FoxP3 positive case.

Results: Twelve cases were ER-Her2+, 27 were ER+Her2-, 25 were ER+Her2+, and 33 were ER-Her2-. The numbers of FoxP3 positive T cells significantly correlated with NG III status (p = 0.00018). The average numbers of FoxP3 positive T cells were 9.5, 19.4, and 27.8 per high powered field for NG I, II, and III, respectively. Using the ≥ 15 FoxP3 positive cells/HPF threshold, 76.9% of NGIII cases were FoxP3 positive compared to 21.0% of NGI cases (p = 0.000021). 74.5% of ER negative cases were FoxP3 positive, compared with 46% of ER positive cases (p=0.0054). Her2 status did not correlate with FoxP3 status (p = 0.71).
Conclusions: The presence of significant numbers (≥15/hpf) of FoxP3 positive Tregs in breast carcinoma was positively associated with higher NG and ER negativity. These results argue that Tregs may play a role in inducing immune tolerance to higher grade, hormone receptor negative breast carcinomas, and are a potential therapeutic target for these more aggressive cancers.

96 Myoepithelial Carcinoma of the Breast: A Clinicopathological and Immunohisytochemical Study of 11 Cases

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Background: Myoepithelial carcinoma of the breast is a rare tumor defined as an infiltrating tumor composed purely of myoepithelial cells (mostly spindled) with mitotic activity. Our knowledge of these tumors is limited.
Design: To further characterize them, we reviewed 11 myoepithelial carcinomas with areas where emanation of tumor cells from the myoepithelial cell layer was confirmed. Cases were retrieved from the consultation files of one of the authors (FT) and from the files at Yale New Haven Hospital from 2003 to 6. Immunohistochemistry for CK5-6, CK903, CD10, p63, SMA, ER, PR, HER2 and EGFR was performed. Clinical details and follow-up information were obtained.
Results: The patients, all female, ranged in age from 43 to 87 years (median 61). Tumor size ranged from 1.3 to 11 cm. All tumors were located within the breast and composed purely of spindle cells with occasional aggregates of more plump epithelioid cells in 3 cases; one case had squamous morules. There was no evidence of an intraepithelial component in any of the cases. All exhibited predominantly grade 1 and rarely focal grade 2 nuclei. Necrosis was absent. All tumors were positive for high molecular weight cytokeratins (CK903, CK5-6) and often for CD10 and p63. SMA positivity was confined to supportive stroma. The stromal component was prominent in 7 cases. All tumors were ER, PR and HER2 negative. EGFR was positive in 3 cases assessed. In all 7 cases with axillary lymph node sampling the lymph nodes were negative. Treatment ranged from lumpectomy to mastectomy with axillary node dissection often followed by postoperative radiation and/or chemotherapy. Follow-up was available for 7 patients (range 7 to 38 months; median 19). Recurrences developed in 2 cases; both interpreted as a reactive stromal change initially and treated with local excision only. At last contact none of the patients had died of disease.
Conclusions: Myoepithelial carcinomas of the breast exhibit the basal phenotype but appear to have an excellent prognosis. Distinction from a reactive stromal change can be a problem. Considering that an intraepithelial carcinoma is absent in the majority of tumors with basal phenotype the existence of tumors with basal phenotype in which the tumor cells can be seen emanating form the myoepithelial cells suggests that breast carcinomas with basal phenotype may arise from myoepithelial cells. The relationship between myoepithelial cells, benign myoepithelial cell proliferations, myoepithelial cell carcinoma and carcinomas with basal phenotype merits further investigation.

97 The Influence of Hormone Receptor Status and HER1 and HER2 Expression on the Response to Neoadjuvant Therapy in Breast Cancer Patients

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Background: To identify the role of estrogen (ER), progesterone (PgR), epidermal growth factor 1(HER1) and epidermal growth factor 2 (HER2) receptors in predicting response and outcome to preoperative chemotherapy.
Design: In a large volume laboratory using standard immunohistochemical methods, we reviewed the pretreatment biopsies and histological specimens at final surgery of 488 patients with large or locally advanced breast cancer (cT2-T4, N0-2, M0) who were treated with preoperative chemotherapy. The incidence of pathological complete remission (pCR) and outcome were assessed with respect to initial pathological findings. ER and PgR status [absent (0% of the cells positive) vs. expressed], HER1 expression [absent (0% of the cells positive) vs. expressed], HER2 expression (overexpressed vs. none) were considered together with other classical prognostic and predictive features.
Results: Patients within the ER/PgR absent cohort were 5.3 times (95% CI 3.20 - 8.85) more likely to achieve a pCR (p < 0.0001). Significant predictors of disease-free survival (DFS) at the univariate analysis were absence of ER and PgR expression (HR 2.7, 95% CI 2.06 to 3.59, p < 0.0001), HER1 expression (HR 1.5, 95% CI 1.03 to 2.29, p = 0.04) and HER2 overexpression (HR 1.58, 95% CI 1.06 to 2.34, p = 0.02). In patients with endocrine non responsive tumors a worse disease-free survival (DFS) was observed if HER2 was overexpressed (HR 1.8, 95% CI 1.10 to 2.84, p = 0.02). A statistically significant difference in DFS was observed at the multivariate analysis only for patients with ER and PgR absent disease (HR: 2.5, 95% CI 1.73 to 3.72, p < 0.0001).
Conclusions: Response to preoperative chemotherapy is significantly higher and outcome significantly worse for patients with ER and PgR absent tumors. HER1 and HER2 may have a prognostic role and might identify patients with poorer outcome within the population with ER and PgR absent tumors. New chemotherapy regimens and combinations with targeted agents should be explored in this cohort of patients.

98 Histologic Associations and Long-Term Cancer Risk in Columnar Cell Lesions of the Breast: A Retrospective Cohort and Nested Case-Control Study

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Background: Columnar cell lesions (CCLs) with atypia have recently become important in view of their observed associations with atypical hyperplasias, ductal carcinoma in-situ, and tubular carcinoma, on both histological and molecular levels. However, a limited number of available retrospective outcome studies have failed to establish an increased risk for recurrence or for the development of invasive cancer on long-term follow-up. The purpose of this study is to assess the long-term cancer risk associated with columnar cell lesions and compare cancer risk among different subgroups of CCLs with and without atypia.
Design: We evaluated the overall cancer risk of the totality of CCLs (817 cases) upon long-term follow-up in 2580 cases from the Nashville Breast Cohort between 1965-83. We also examined 241 cases of CCLs and classified them into three different categories based on Schnitt and Vincent-Salomon's classification scheme -CCLs without hyperplasia, CCLs with hyperplasia lacking atypia, and CCLs with atypia *(Adv Anat Pathol. 2003 May;10(3):113-24)*. We then compared, in a nested case-control design, the risks associated with the three different categories of CCLs. Cases were those women who subsequently developed invasive cancer beyond six months after their entry biopsy. Two controls who did not develop cancer were selected for each case, matched by age at biopsy and year of biopsy. Relative risks of breast cancer were estimated by odds ratios derived from conditional logistic regression analyses.
Results: Our results indicate a mild increase in the overall cancer risk associated with the totality of CCLs (RR=1.72 at 10 years) and no significant difference among the three categories of CCLs with regards to future cancer risk. We did observe, however, a 2-3 fold increase in the incidence of atypical hyperplasias when CCLs were present versus when they were lacking in the benign breast biopsy.
Conclusions: The results highlight that, although an observational association is present between columnar cell lesions and atypical hyperplasia, the precursor nature of these lesions is not well supported yet, and evidence of high cancer risk is lacking. The implications of such a finding on benign breast biopsy should be that of a possible concomitant more worrisome lesion, rather than one of a significantly increased long-term breast cancer risk.

99 Breast Cancer in Women from Ghana and What It May Reveal about Breast Cancer in African-American Women

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Background: In the USA approximately 40,000 people die each year of breast cancer. Women of African decent are diagnosed with breast cancer less frequently than Caucasians. However, breast cancer in African-American women is diagnosed at an earlier age and carries a higher mortality rate. Socioeconomic issues may contribute to this phenomena, but they clearly don't account for all cases. We hypothesize that distinct genetic alterations may result in distinct histological and immunophenotypic differences in breast cancers from women of African descent. In this study we have evaluated a cohort of invasive breast cancers from women in Ghana, a region of Africa that has a genetic link to most African-American women in The United States.
Design: We have obtained unselected deidentified 24 invasive breast cancers form women in Ghana. We have evaluated these tumors histologically and immunohistochemically based on the following criteria: Tumor type, Nottingham grade, presence of necrosis, angiolymphatic invasion, presence of central scar, micropapillary architecture, presence of lymphocytes, concurrent ductal carcinoma in-situ, estrogen receptor status, progesterone receptor status and Her2Neu overexpression.
Results:

Data Summary

BR=3	ER Neg	PR Neg	H2Neu Neg	Micropapillary	Central Scar	Necrosis	Angiolymphatic Inv.
20/24	20/24	19/24	22/24	8/24	8/24	12/24	7/24

BR=Modified Bloom-Richardson, ER= estrogen receptor, PR= progesterone receptor

The data showed that 83% of randomly identified invasive carcinomas were high grade (modified Bloom-Richardson grade 3). 58% were immunophenptypically triple negative (ER, PR and Her2neu).
Conclusions: Invasive breast cancers from African women from Ghana are commonly triple negative and of high histological grade. Tumor necrosis and micropapillary architecture are common histological features. These features are comparable to tumors from women who are proven to have the BRCA1 and BRCA2 gene mutation. It is our conclusion that these results are suggestive of a genetically unique breast cancer occurring in African women. Furthermore, the genetic link that exist between women from Ghana and African American women should lead to further study in identifying a unique genetic marker to predict for this very aggressive form of breast cancer.

100 Is p120 as Effective as E-Cadherin (Ecad) in Distinguishing Lobular (L) from Ductal (D) Carcinomas of the Breast? A Study of 235 Cases

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Background: Similar to Ecad, the immunostain against p120 which anchors Ecad to the actin cytoskeleton has been found to be helpful in distinguishing mammary L from D carcinomas (Dabbs, et al. AJSP, 2007). We surmised that a positive stain [p120] would be easier to interpret than a negative (-) stain [Ecad] and tested our hypothesis on tissue microarrays (TMAs) of known cases of invasive and in-situ L and D carcinomas.
Design: 4-µm TMA sections of 131 poorly-differentiated invasive D carcinomas (IDC), 44 pleomorphic invasive L carcinomas (pILC), 19 florid L carcinoma in-situ (fLCIS), and 41 D carcinoma in-situ (DCIS) with low (15), intermediate (12) or high (14) nuclear grade were utilized. pILC and fLCIS were selected due to their greater

morphologic similarity to D than classical L carcinomas. Immunohistochemistry using the Bond Polymer Refine Detection Kit (Vision BioSystems) and monoclonal antibodies against p120 (BD Transduction Labs, San Jose, CA) and Ecad (Invitrogen; Carlsbad, CA) was performed. A L phenotype was defined as either loss or weak, discontinuous cell membrane staining (memb+) for Ecad and/or strong cytoplasmic staining (cyto+) for p120. Strong memb+ for Ecad and/or p120 defined a D origin.
Results: 44/44 (100%) of pILC were Ecad - while 39/44 (87%) showed strong p120 cyto+. 5 showed weak p120 cyto+. 19/19 (100%) of fLCIS were Ecad - and p120 cyto+. Of 15 low-grade DCIS, 14/15 (93%) showed strong memb+ for both Ecad and p120. 1 showed weak memb+ for both. 12/12 (100%) intermediate-grade DCIS showed strong Ecad and p120 memb+. 13/14 (93%) high-grade DCIS showed strong Ecad memb+ while 1 case showed weak, discontinuous memb+. For p120, 14/14 (100%) showed strong memb+. Of the 131 IDC, 90 (69%) showed strong memb+ for both Ecad and p120. 15/131 (12%) showed strong Ecad memb+ but weak p120 memb+. Conversely, 12/131 (9%) showed weak Ecad memb+ but strong p120 memb+. 7/131 (5%) showed weak memb+ for both. 6/131 (5%) were reclassified as L (5 pleomorphic L, 1 D with L features) after both stains were consistent with a L phenotype and morphologic re-review. 2 cases were ambiguous where stain results were discordant with morphologic features.
Conclusions: p120 and Ecad were equally effective in distinguishing L from D carcinomas. However, weak memb+ for p120 occurred in 10% of D cases which could be misinterpreted as a L phenotype whereas no L cases showed strong memb+ for Ecad. Ecad may be a superior stain than p120 if used alone, however, utilizing both is the best diagnostic strategy.

101 The Novel Estrogen-Induced Gene EIG121 Is Over-Expressed in ER-Positive Breast Cancers and Promotes Autophagy

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Background: The novel estrogen-induced gene EIG121 was identified from a microarray analysis of postmenopausal women taking hormone-replacement therapy. EIG121 is over-expressed in estrogen-dependent endometrioid endometrial carcinoma, particularly the lower grade tumors. EIG121 has been shown to be the single best genetic identifier that distinguishes estrogen-dependent endometrioid tumors from estrogen-independent, non-endometrioid tumors. Therefore, we have hypothesized that EIG121 has a critical function in the pathogenesis of endocrine-related tumors. The normal cellular function of EIG121 is unknown, as its expression pattern in another estrogen-dependent organ, the breast.
Design: We performed qRT-PCR analyses of EIG121 using 30 different breast tissues, including 10 normal breast samples, 10 ER positive breast carcinomas, and 10 ER negative breast carcinomas. Dual immunofluorescence was performed to ascertain the subcellular compartment of EIG121. Enforced over-expression of EIG121 and siRNA-mediated knock-down of EIG121 were performed to determine EIG121's effect on cell proliferation.
Results: EIG121 was significantly over-expressed in ER positive breast carcinomas compared to normal breast and ER negative breast carcinomas. Dual immunofluorescence demonstrated that EIG121 localized to the membranes of the TGN-late endosome-lysosome compartments. Knock-down of EIG121 significantly sensitized the cells to serum withdrawal, causing massive apoptosis. Enforced over-expression of EIG121 in a tetracycline-inducible system caused decreased cell growth and the accumulation of cytoplasmic vacuoles. However, we did not observe an increase in apoptotic or necrotic cell death. By electron microscopy, the accumulated cytoplasmic vacuoles had double or multiple membranes and contained recognizable cellular organelles, which is highly characteristic of autophagy. LC3 immunofluorescence demonstrated numerous punctate structures in the cytoplasm of EIG121 over-expressing cells, which is also characteristic of autophagy.
Conclusions: Autophagy is a cellular pro-survival mechanism in unfavorable growth conditions such as nutrient deprivation, growth factor withdrawal, and hypoxia. Similar conditions are encountered by tumorigenic cells in the center of a developing tumor. The evidence presented here supports the hypothesis that EIG121 is involved in promoting cell survival, likely via promoting autophagy. The link between hormone responsiveness and autophagy is unknown at this time. It is possible that lysosomal-based EIG121 regulates signaling through ER.

102 A Prospective Study Comparing SP1 and 1D5 Monoclonal Antibodies to the Estrogen Receptor Reveals Similar Staining Results in Routine Clinical Use

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Background: Rabbit monoclonal antibody (MAb) SP1 has an 8-fold higher affinity for estrogen receptor (ER) protein than the commonly used mouse MAb 1D5. In a recent study comparing immunohistochemical staining for SP1 and 1D5 in over 4000 invasive carcinomas, 8% of cases were found to be SP1 positive and 1D5 negative (J Clin Oncol 24:5637-44, 2006). The implication of this study is that 1D5 may fail to identify a group of women who might benefit from endocrine therapy. However, the tissue samples from this study had all been previously frozen prior to formalin fixation and 97% were examined by tissue microarray.
Design: A prospective study compared SP1 and 1D5 immunostaining on consecutive cases of breast carcinoma analyzed for routine clinical care during the study period. All cases were received fresh, then formalin fixed and paraffin embedded. Immunohistochemistry was performed on 5 micron sections according to manufacturer recommendations. Slides were scored for ER positivity by two pathologists using the following categories: <1% negative; 1-10% positive-low; >10% positive.
Results: In total, 164 cases were analyzed, of which 116 (71%) cases were invasive carcinomas, 38 (23%) were DCIS, and 10 (6%) were metastases. 38 (23%) cases tested were ER negative by both 1D5 and SP1 (29 cases of invasive carcinoma, 6 cases of DCIS, and 3 metastases). Only 1 case (0.6%) with discrepant staining results was identified: an invasive carcinoma from an excision. For this case, 1D5 received a negative score as only rare cells (<1%) showed immunoreactivity. However, SP1 scored as positive-low as 5-10% of cells showed weak positivity. Also noted was cytoplasmic staining by 1D5 in 5 (3%) ER-negative cases (4 invasive carcinomas and 1 metastasis). Cytoplasmic positivity was never observed with SP1.
Conclusions: In this prospective comparison of SP1 and 1D5, only 1 breast carcinoma out of 164 (0.6%) showed discrepant results: "positive-low" with SP1 and "negative" with 1D5. Thus, these MAbs give very similar results in tissue from routine clinical samples that have not been previously frozen. These findings support the use of either antibody for routine clinical practice with discrepant results in <1% of cases.

103 Clinicopathologic Analysis of ER/PR Positive and HER2/*neu* Positive Breast Carcinoma

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Background: HER2/*neu* gene amplification and/or overexpression has been shown to be inversely corraleted with estrogen receptor (ER) expression in breast carcinomas (BC). However, HER2/*neu* is overexpressed in a small, but significant portion of ER positive tumors. Several studies have shown that this group of BC are less likely to respond to hormonal therapy compared to ER positive HER2/*neu* negative tumors. Despite the clinical importance of this group of BC, their clinicopathologic features have not been described in detail.
Design: We selected 374 invasive BC for the study. All H&E slides were reviewed and the diagnoses confirmed including histological type and grade. ER and progesterone receptor (PR) status were assessed by immunohistochemistry (IHC). Tumors showing at least 10% nuclear staining were considered positive. HER2/*neu* status was determined by IHC (n=225), FISH (n=23) or both IHC and FISH anaysis (n=126). Cases showing 3+ membrane immunoreactivity by IHC or gene amplification (HER2/CEP17 ratio >2) by FISH were considered positive.
Results: Of the 374 cases 308 (82.4%) were ER/PR positive HER2/*neu* negative, 34 (9.1%) were ER/PR positive HER2/*neu* positive and 32 (8.5%) were ER/PR negative HER2/*neu* positive. The histopathologic features of the three groups are summarized in Table 1.

Summary of clinicopathologic features

		ER/PR+, HER2-	ER/PR+, HER2+	ER/PR-, HER2+	p
Age	Median	59	50	48.5	< 0.0001*
	Mean ± SEM	58.6 ± 0.7	50.6 ± 1.8	48.9 ± 2.1	
Size	Median	1.5	2.1	2.0	0.0034*
	Mean ± SEM	2.0 ± 0.1	2.5 ± 0.3	2.8 ± 0.4	
Histologic type	Ductal	242	33	32	0.0045**
	Mixed	12	1	0	
	Lobular	54	0	0	
Histologic grade	Low	92	2	0	< 0.0001**
	Intermediate	177	18	7	
	High	39	14	25	
Mitoses per 10 hpf	Median	3	12	25	< 0.0001*
	Mean ± SEM	6.5 ± 0.6	15.8 ± 3.7	28.7 ± 4.4	
Lymphovascular invasion	Absent	224	15	18	0.0012**
	Present	84	19	14	
Lymph node status	pN0	163	17	12	0.0097**
	pN0(i+)	20	1	0	
	pN1mi	23	3	0	
	pN1a-3	82	13	20	
Race	Caucasian	251	22	21	0.0038**
	Non-caucasian	33	9	8	
Presentation	Palpable mass	136	19	21	0.0382**
	Screening	172	15	11	

* Kruskal-Wallis test, ** Chi-square test

Conclusions: Our results suggest that ER/PR positive BC showing HER2/*neu* overexpression/gene amplification have clinicopathologic features more similar to ER/PR negative HER2/*neu* positive rather than ER/PR positive HER2/*neu* negative tumors.

104 Stem Cells (CD44+/CD24- or low) Are Differentially Expressed in Triple Negative (ER-, PR- and Her2-) Invasive Mammary Carcinoma Phenotype

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Background: Breast cancer stem cells, defined as CD44(+)/CD24(-/low), are thought to be slowly cycling cells, and as a result, they may escape targeted intervention when not actively proliferating. This may be an important reason behind breast cancer treatment failures and recurrences. The purpose of the study is to evaluate the expression of CD44 and stem cells in tumors that are negative for estrogen receptors, progesterone receptors and Her2 (triple negative phenotype).
Design: Fifty archival formalin fixed paraffin embedded tissues containing infiltrating mammary carcinoma were randomly selected. The cases were categorized into those with triple negative phenotype, those with non-triple negative phenotype and those with known recurrence. The immunohistochemistry stains, CD44 and CD24, were performed on all specimens. The intensity (0, 1+, 2+, 3+) and percentage (0-100%) of tumor cell staining were independently evaluated by 2 observers, discrepant results were resolved by reviewing the cases together and agreeing on the scores. Positive staining was defined as > 5% staining of tumor cells at ≥2+ intensity. Cases with CD44 positive and CD24 negative (CD44+/CD24-) were considered as having stem cells. At least a ratio of 4:1 CD44:CD24 was required for CD44+/CD24 low. Chi square was used in the statistical evaluation of the data.

Results: There were 16 (32%) cases with triple negative phenotype and 34 cases (68%) with non triple negative phenotype. There were 12 (24%) cases with recurrence. Stem cells (CD44+/CD24 – or low) were present in 10 of 16 (62.5%) triple negative phenotype but present in 8 of 34 (23.5%) non triple negative phenotype cases. Of the 12 cases with recurrent carcinoma, 8 (66.7%) expressed stem cells.

Conclusions: Tumor stem cells appeared more significantly expressed in breast carcinoma cases with triple negative phenotype. There also appeared to be more stem cell expression in cases with recurrence. The significant expression of stem cells may not be independent and may in fact be related to the hormone receptor status.

Stem cells and triple negative invasive mammary carcinoma phenotype

	Stem cell + (n=18)	Non-stem cells (n=32)
Triple negative (n=16)	10 (62.5%)	6 (37.5%)
Non-triple negative (n=34)	8 (23.5%)	26 (76.5%)

P value = 0.000531

105 Subcellular Localization and Patterns of Expression of Fibroblast Growth Factor Receptor-1 (FGFR1) and Its Phosphorylated Form FGFR1-pY^{653} in Human Breast Cancer

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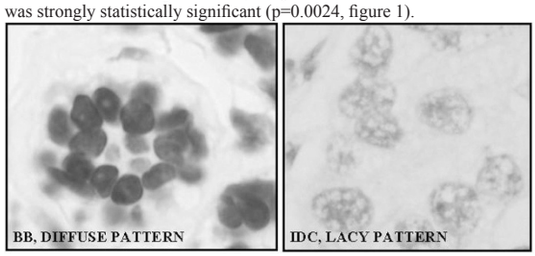
Background: FGF/FGFR contribute to the induction, progression and metastatis of breast cancer. Although aberrant expression of FGF/FGFR was noted in breast carcinomas it is unclear if and how amplification of FGF/FGFR loci contribute to inappropriate activation of associated signaling pathways leading to malignant progression.

Design: We analyzed expression of FGFR1 and p-FGFR1-pY^{653} in benign and malignant breast by immunohistochemistry using tissue microarrays. We stained 8 cases of benign breast (BB), 29 and 24 ductal carcinomas *in situ* (DCIS), as well as 38 and 36 invasive ductal carcinomas (IDC) for FGFR1 and p-FGFR1-pY^{653} respectively. The preferential expression of FGFR was coded as nuclear (N), cytoplasmic (C), or equal (E) and the intensity of FGFR as strong (S), weak (W) or absent (O). The expression of p-FGFR1-pY^{653} was nuclear and the staining pattern was lacy, granular or diffuse. Two pathologists independently reviewed the staining pattern. The results were analyzed by Fisher exact test.

Results: FGFR1 expression was predominantly nuclear in 55% of IDC, 45% of DCIS and 25% of BB. It was predominantly cytoplasmic in 29% of IDC, 45% of DCIS and 63% of BB. There was no preferential expression in 16% of IDC, 10% of DCIS and 12% of BB. This difference in the localization of FGFR1 expression was not significant (p=0.37). Likewise, the intensity of FGFR1 expression was not statistically different (p=0.83). FGFR-pY^{653} was present in the nuclei of all groups. The lacy pattern was seen in 39% of IDC, 42% of DCIS and none of BB. The granular pattern was found in 50% of IDC, 50% of DCIS and 25% of BB. Only 11% of IDC, and 8% of DCIS showed diffuse nuclear staining. However, this pattern was noted in 75% of BB. The difference was strongly statistically significant (p=0.0024, figure 1).



Conclusions: FGFR1-pY^{653} localizes exclusively to the nuclei of all groups. The pattern of FGFR1-pY^{653} expression is distinctly different between malignant and benign breast tissue. This may be a reflection of the distinct biological role that FGFR1 has in benign and malignant breast epithelium.

106 Serial Analysis of Gene Expression of Lobular Carcinoma In Situ Identifies Upregulation of Metalloproteinase MMP-9 and Downregulation of Tight Junction Protein Claudin 4

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Background: Global gene expression analysis is difficult to perform on lobular carcinoma in situ (LCIS) of the breast, because most lesions are incidental microscopic findings that are too small to yield adequate RNA.

Design: We obtained frozen tissue from a rare index case of mass-forming (6 cm) LCIS with ductal extension and focal necrosis (so called florid LCIS). By immunohistochemistry (IHC), we confirmed loss of E-cadherin protein in the LCIS. Frozen tissue was macroscopically dissected to enrich for lobules distended by LCIS, and total RNA prepared using the guanidium CsCl protocol. 20ug RNA was used to create a long-serial analysis of gene expression (SAGE) library. The LCIS SAGE library was compared to SAGE libraries of normal duct epithelial cells to identify genes differentially expressed by 3 fold, and a SAGE library of breast stroma to eliminate genes likely representing stromal contamination. Promising over- and underexpressed candidate genes were validated by IHC on a series of formalin-fixed, paraffin embedded cases of LCIS.

Results: Compared to normal epithelium, 353 expressed sequence tags were differentially expressed in LCIS (209 overexpressed and 144 underexpressed by > 3 fold). Overexpressed genes of interest included matrix metalloproteinases (MMP) 2, 9, and 13, S100A4, HSP 90, and amyloid beta precursor protein. Underexpressed genes included E-cadherin (consistent with the IHC results on the index case), cadherin associated proteins alpha and delta catenin, claudins 1, 3, and 4, and syndecan 4. By IHC, Claudin 4 was underexpressed in 9 of 11 cases of LCIS compared to normal luminal cells. MMP-9 was overexpressed in 15 of 19 cases of LCIS compared to normal luminal cells. In contrast, MMP-2 expression was similar between LCIS and normal luminal cells in 8 of 9 cases. Quantitative RT-PCR confirmed the latter differences between dissected LCIS and normal epithelium in the index case; while MMP-9 mRNA levels were 7 fold increased in the LCIS, MMP-2 levels were not increased.

Conclusions: SAGE identifies altered genetic pathways in LCIS, some of which may represent therapeutic targets. The unexpected overexpression of an invasion-promoting protein such as MMP-9 in LCIS, a lesion considered either a low-risk precursor or a generalized marker of breast cancer risk, invites further study.

107 High Interlaboratory Reproducibility of the Silver In Situ Hybridization Assay (SISH) for the Detection of *HER2* Gene Status in Breast Carcinoma: A Study Reporting Results from Five Cancer Institutes

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Background: Dual-color fluorescence in situ hybridization (FISH) is the gold standard technique for the assessment of the human epidermal growth factor receptor 2 (*HER2*) gene amplification in breast cancer. Recent studies have illustrated a highly sensitive enzyme metallographic silver in situ hybridization technique (SISH), which has been successfully employed in the assessment of the state of *HER2*. Specific aims of this study were: 1) to demonstrate interlaboratory feasibility of the *HER2* SISH assay on samples from five sites; 2) to evaluate interlaboratory reproducibility of the *HER2* SISH assay at five laboratories; and 3) to compare SISH results with other ISH methods.

Design: The *HER2* gene status of 89 breast carcinomas was analyzed in parallel by using direct-labeled manual FISH, manual chromogenic ISH (CISH™) and bright field automated SISH. Evaluation was carried out by pathologists at five different laboratories according to the algorithms provided by the manufacturers' instructions and the guidelines of the American Society of Clinical Oncology/College of American Pathologists. Reproducibility was evaluated by computation of the weighted kappa statistic.

Results: Overall results of the study demonstrated interlaboratory feasibility of the *HER2* SISH assay on samples from five sites, in terms of assessment of efficacy and robustness, and interlaboratory reproducibility proved to be satisfactory (Kw=0.91). Moreover, there was a significant association between SISH, CISH™ and FISH in regard to HER2 status assessment, as well as between FISH and CISH.™ Discrepancies were observed primarily in tumors with aberrant ISH patterns and intra-tumor heterogeneity of *HER2* gene amplification.

Conclusions: A good rate of concordance among the ISH methods for assessing *HER2* status was observed. In this context, SISH can be regarded as a "value-added" tool because it is fully automated and highly reproducible between laboratories.

108 Novel Rabbit Monoclonal Antibody SP3 Demonstrates Excellent Concordance with Flourescence In-Situ Hybridization (FISH) in 262 Invasive Breast Carcinomas and Is a Reliable Antibody To Assess HER-2/neu (H2N) Status

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Background: The novel rabbit monoclonal antibody SP3 targeted against the extracellular domain of HER2 has been reported to demonstrate a high level of agreement when compared to the antibody CB11 or by chromogenic in situ hybridization (Ricardo, S et al. J Clin Pathol, 2006) in tissue microarrays. However, FISH is considered the gold standard to assess H2N status in breast cancer patients. The goal of this study was to compare H2N detection by immunohistochemisty (IHC) using the SP3 antibody with FISH results in whole tissue sections.

Design: Parallel four micron-thick whole tissue sections from 262 invasive breast carcinomas were used to perform IHC using the Bond Polymer Refine Detection Kit (Vision BioSystems) with monoclonal antibody against SP3 (Labvision Corp.-NeoMarkers, Fremont, CA) and FISH with the HER-2 DNA probe kit (Vysis/Abbott Molecular, IL). H2N protein expression was blindly scored on a scale from 0-3+ according to the 2007ASCO/CAP guidelines. H2N gene amplification was defined as HER-2:CEP 17 signal ratio of greater than 2.2. Results of IHC and FISH were compared in each case.

Results: Of 262 cases, 136 showed 0, 79 showed 1+, 12 showed 2+ and 35 showed 3+ staining, respectively. All cases demonstrating 3+ staining were FISH positive (+). 2+ cases constituted 5% of all cases and demonstrated variable FISH amplification (8 of 12 cases were FISH +). Discordance of staining with FISH results was demonstrated in 10% of all cases, exclusively within the SP3 IHC 0 and 1+ subgroups, of which 64% of FISH + cases corresponded to a low amplification ratio (2.3-3.3). FISH+ was inadvertently determined from in-situ carcinoma cells in 3 cases (1%).

Conclusions: SP3 demonstrates excellent concordance with FISH with 100% agreement in 3+ cases. In addition, 2+ cases only constituted 5% of all cases. The SP3 immunostain is easy to interpret with minimal, if any, non-specific, background staining and can be reliably used to assess H2N status in breast cancer patients.

109 The Presence and Extent of Retraction Artifact and Micropapillary Features on Core Needle Biopsy Specimens Predict Lymph Node Metastasis in Breast Carcinoma

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Background: Nodal metastasis is the first sign of disseminated disease and an established poor prognostic factor in breast carcinoma. Breast carcinomas with micropapillary (MP) features were commonly associated with nodal metastasis in prior studies. We have recently reported that the presence and extent of retraction artifact (RA) in breast cancers without MP features is also a strong predictor of nodal metastasis. We examined whether the presence and extent of MP features and RA in core needle biopsy (CNB) material can be used to predict lymph node metastasis in patients with breast cancer.

Design: We selected 30 cases of invasive ductal carcinoma with MP features (IMPC) and 326 cases of invasive ductal carcinoma without MP features (IDC). CNB and subsequent excisional biopsy were available in all cases. All H&E slides were reviewed and the presence and extent of MP features and RA were determined. Results obtained in the CNB and corresponding excisional biopsy were compared and correlated with clinicopathologic features and presence of nodal metastasis.

Results: MP areas were present on CNB in 16 of 30 (53%) IMPC cases ranging in extent between 10-60%. Nodal metastases were found in 16 of 16 (100%) and 12 of 14 (86%) IMPC cases with and without MP features present on CNB, respectively. While the extent of MP features on excisonal biopsy was significant higher in the former group, its extent did not correlate with the presence of nodal metastasis. In the cohort of IDC we found a highly significant correlation between the extent of RA present in CNB and corresponding excisional biopsy ($r=0.5666$, $p<0.0001$). The presence of extensive RA (≥20% tumor area) also showed highly significant association with the presence of nodal metastasis both on CNB (RR=1.87, 95% CI 1.44-2.43, $p<0.0001$) and excisional biopsy (RR=2.80, 95% CI 1.99-3.93, $p<0.0001$) material. The extent of retraction artifact in IDC showed highly significant correlation with tumor size, grade, lymphatic invasion and HER2 status.

Conclusions: Our results suggest that the presence of MP features or extensive RA on CNB of breast carcinoma can predict the presence of nodal metastasis. The significant correlation between the extent of RA observed on core and excisonal biopsy provides support to the hypothesis that this phenomenon is more than just an "artifact" of fixation and confirms our previous results in a new, independent cohort of patients.

110 GATA-3 as a Marker of Recurrence and Survival in Breast Cancer

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Background: Gata-3 is a transcription factor closely associated with estrogen receptor alpha (ER) effect in breast carcinoma. Decreased expression of Gata-3 is associated with resistance to hormonal therapy. We investigated the immunohistochemical expression of Gata-3 in ER positive and ER negative breast carcinomas. Time to recurrence and death were the two main endpoints of this analysis.

Design: One hundred sixty six cases of invasive breast carcinomas with 10-year follow-up information were included. All cases were treated with surgery, hormonal therapy (ER positive patients) and chemotherapy. Positive Gata-3 and ER cases were defined as greater than 20% of cells staining. Time to cancer recurrence and time to death were analyzed with survival methods (Kaplan-Meier and Cox proportional hazards regression).

Results: A total of 38 (23%) patients tumors recurred, with a median time to recurrence of 19.5 months, while 51 (31%) deaths were observed with a median time from surgery to death of 36.5 months. 97 cases were Gata-3 positive and 69 were negative. Gata-3 positive tumors were associated with substantially lower risk of recurrence (hazard ratio, HR = 0.52, p = 0.041). The mortality risk was lower in Gata-3 positive (hazard ratio, HR = 0.75, p = 0.310) than Gata-3 negative tumors. Of the 38 ER negative and Gata-3 negative cases, 14 (37%) and 15 (39%) died. There were no ER negative and Gata-3 positive patients. Of the ER positive cases, 28 were Gata-3 negative and 93 were Gata-3 positive. Among the ER positive Gata-3 negative tumors, there were 7 (25%) recurrences and 8 (29%) deaths. In contrast, among the ER positive Gata-3 positive tumors, there were 16 (17%) recurrences and 24 (26%) deaths. Thus, controlling for ER expression (ER positive cases only), Gata-3 positive tumors had a lower recurrence (HR = 0.66, p = 0.363) than Gata-3 negative tumors. However, mortality was not significantly decreased (HR = 0.87, p = 0.728) in patients with ER positive, Gata-3 negative tumors.

Conclusions: Our data suggest that patients with estrogen receptor alpha positive tumors with decreased Gata-3 expression have a shortened interval to recurrence and death, perhaps because of reduced response to hormonal therapy. Within patients with ER positive tumors knowing the status of Gata-3 may help identify patients that are more likely to benefit or not from hormonal therapy.

111 Fixation Issues with Breast Carcinoma Hormone Receptors: ER Negative PR Positive Carcinomas Exist Even with Optimal Fixation Methods

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Background: There is renewed interest in the fixation of tissues for the immunohistochemical detection of prognostic and predictive markers for purposes of test accuracy and reproducibility. There is some suggestion in the literature that false negative ER results may occur as a result of inadequate formalin fixation times, and that the category of ER negative/PR positive cases reflect this issue. The recent ad-hoc committee on IHC standardization published guidelines for IHC in general (Goldstein N et al Recommendation for improved standardization in immunohistochemistry Appl Immunohistochem Mol Morph 2007 June 15(2) 124-133) and the group now recommends eight hours as the minimum exposure time to formalin for ER/PR receptors for breast carcinomas (manuscript in press).

Design: The quality records of breast cancer hormone results were reviewed for the time periods Jan 2005-Dec 2005 (902 cases), Jan 2006-Dec 2006 (1019 cases), and Jan 2007-June2007 (524 cases). ER antibody clone 6F11 and PR clone 1A6 were used according to IVD protocol on the Benchmark XT (Ventana, Tucson, AZ). The 2000 NIH consensus conference on breast hormone receptors forms the basis of reporting at our institution. Any nuclear immunostaining is a positive result and is semi-quantitated with intensity and proportion of immunostained cells. Hormone receptors were performed predominately (98%) on core biopsies. Prior to Jan 2007, the precise exposure time to formalin was not recorded. Beginning Jan 2007, all specimens (cores and resections) were exposed to formalin a minimum of 8 hours and not more than 48 hours.

Results: The following table reflects percentages in each column:

Table 1

	2005	2006	2007
All ER+	79.8	79.7	83
ER+PR+	71.8	72.8	72.5
ER+PR-	8.0	6.9	10.5
ER-PR+	5.4	5.7	5.7
ER-PR-	14.8	14.6	11.3

Conclusions: (1) Controlled formalin fixation resulted in a small increase of (weakly) ER+ cases of 3.2% year to date in 2007. (2) The increase number of (weakly) ER+ cases was almost exclusively confined to the ER+/PR- subgroup. (3) The percent of ER-/PR+ cases was unchanged in time across this series, indicating that under-fixation for ER was not an issue affecting the result, and that the ER-/PR+ subgroup, using antibody clones 6F11 and 1A6, is not an artifact of fixation. (4) Formalin fixation needs to be controlled according to recent guidelines for optimizing predictive marker outcomes.

112 Immunohistochemical Profile in Neuroendocrine Carcinoma of the Breast

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Background: Neuroendocrine carcinoma of the breast (NECB) is an uncommon neoplasm that accounts for only 2-3% of primary breast cancers. Presence of frequent dimorphic histologic appearance has been also reported in this tumor. The aim of our study was to investigate immunohistochemical markers known as epithelial breast tumor markers, neuroendocrine/small cell carcinoma markers and prognostic markers in the NECB.

Design: Nine cases of NECBs: 3 cases from The Methodist Hospital Pathology Department files, and 6 cases from Asan Medical Center (AMC) were reviewed. Immunohistochemical staining of molecular markers were performed, including neuroendocrine/ lung small cell carcinoma markers (chromogranin, synaptophysin, CD56, and TTF-1), basal phenotype markers (cytokeratin (CK) 5/6, CK 14), prognosis markers (Her-2, p53, Mib-1, EGFR, MUC-1, and C-kit), and hormone receptor markers (ER, PR).

Results: The frequency of neuroendocrine markers expression was found for CD56 in 100%, synaptophysin in 89%, and chromogranin in 33%. TTF-1 was negative in all 9 cases. Basal phenotype (CK5/6 and CK14) was demonstrated in 1(11%) case. ER and PR were expressed in 44% and 22% cases, respectively. EGFR and Her-2 were negative in all 9 cases. MUC-1 and C-kit were expressed in 33% and 11% cases, respectively.

Immunohistochemical Profile in Neuroendocrine Carcinoma of Breast

	Chromogranin	Synaptophysin	CD56	TTF-1	CK5/6	CK14	Her-2
Postitive case(%)	3/9(33%)	8/9(89%)	9/9(100%)	0/9(0%)	1/9(11%)	1/9(11%)	0/9(0%)
	ER	PR	p53	Mib-1	EGFR	C-kit	MUC-1
Positive case(%)	4/9(44%)	2/9(22%)	1/9(11%)	75%	0/9(0%)	1/9(11%)	3/9(33%)

Conclusions: 1) CD56 and synaptophysin showed a higher frequency of expression than chromogranin in NECB. 2) TTF-1 appeared to be a good differential marker from lung small cell carcinoma. 3) One case of NECB showed basal phenotype, but Her-2 and EGFR were not expressed in all 9 cases of NECB. 5) Hormone receptor positivity appeared to be lower in NECB than that of overall breast cancers. These results may be important for the management of NECB and await further studies.

113 Isolated Tumor Cells on Sentinel Lymph Node Biopsy: Our Experience over a Decade

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Background: The use of sentinel lymph node biopsy (SLNB) has essentially replaced complete axillary lymph node dissection for axillary staging of patients with breast cancer. The sixth edition of the American Joint Committee on Cancer (AJCC) staging system for breast cancer included revisions to distinguish between micrometastasis and isolated tumor cells (ITC) and also incorporated the use of immunohistochemistry in breast cancer staging. ITCs, defined as foci less than 0.2 mm in size identified either by histologic examination or immunohistochemistry, are pN0(i+). Completion axillary dissection in this group of patients is controversial as the presumed risk of metastasis to the non-sentinel lymph nodes (NSLN) appears to be very low. We reviewed our data for completion axillary dissection for patients with ITC in the sentinel lymph node.

Design: We reviewed cases of SLNB that had identified isolated tumor cells either by histologic examination or immunohistochemistry. ITC was defined as foci of single cells or small aggregates of tumor cells that measured between 0 and 0.2 mm. We reviewed the presence or absence of tumor in the non-sentinel lymph nodes for patients who underwent axillary dissection.

Results: 1993 patients from June 1997-August 2007 underwent SLNB. Of these, 431(22%) were considered positive including 83 patients whose SLNB contained ITC. 41 patients (49%) underwent axillary lymph node dissection with an average of 15 nodes removed (range 6-39). ITCs occurred as clusters in 12(29%) and as single cells in 29(71%). ITCs were identified by immunohistochemistry in all cases but by routine histochemistry in only 9(22%). In 3 (7%) patients, metastasis was noted in the NSLNs. Comparison of cases with and without non-sentinel node metastasis are presented in Table 1.

	Mean tumor size (cm)	Grade	LVI*
Positive NSLN	1.97	MD(33%);PD(67%)	66%
Negative NSLN	1.77	WD(7%);MD(68%);PD(24%)	13%

*p=0.07;WD:Well-differentiated; MD:Moderately differentiated; PD:Poorly differentiated

Conclusions: The presence of NSLN metastasis in patients with ITC is not negligible, noted in 7% of cases in our study. Higher grade and the presence of lymphovascular invasion (LVI) may play a role in determining which patients require completion axillary dissection.

114 Effect of ASCO/CAP Recommended Guideline for Positive Her2/neu Stain on FISH Concordance – An Evidence Based Study

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Background: Accurate identification of breast cancers overexpressing Her2/neu (Her2) is critical for identifying patients most likely to respond to agents that target Her2, such as trastuzumab, while minimizing the potential impact of serious treatment-associated side effects. The recently developed ASCO/CAP guidelines for Her2 staining by immunohistochemistry (IHC) have raised the definition for a positive result to strong uniform membrane staining (3+) in >30% of invasive cancer cells from 2+ staining in >10% cells. This evidence based study was aimed to determine the effect of raising the cut off level for a positive IHC result on its concordance with FISH positivity.

Design: A total of 719 consecutive cases of primary and metastatic invasive breast carcinomas were selected from our files over a 2 year period. Her2 IHC was performed with the PathwayHer2 antibody from Ventana using the Benchmark automated stainer and analyzed using the Ariol Scan system by Applied Imaging Corp. All the cases were also reviewed independently by two pathologists and the Her2 staining intensity and percent positivity were recorded. FISH analysis was performed by the PathVysion kit and read manually. Staining intensity and percent positivity in invasive cancers was correlated with FISH results and evaluated using descriptive statistics.

Results: 12.1% of cases were IHC+ whereas 15.4% cases were FISH+. 95.4% IHC+ cases were FISH+ and 98% IHC negative cases were FISH negative. FISH positivity dropped sharply in the IHC equivocal group irrespective of percent positivity in these cases (Table 1). In the FISH+ cases, the Her2/Cep17 ratios for IHC+ cases varied between 2.2 to 33.7 (median 11.8) while that for the IHC equivocal cases varied between 2.2-14.2 (median 4.3). The sensitivity and specificity of IHC assay (excluding equivocal cases) was 91.2% and 99.5% respectively.

Table 1: Results of IHC and FISH.

		Total cases (%)	FISH >2.2	FISH 2.2-1.8	FISH <1.8	% FISH+
IHC positive	3+>30%	87 (12.1)	83	1	3	95.40
IHC equivocal	2+>50%, 3+<30%	37(5.1)	8	1	28	21.60
IHC equivocal	2+, 10-50%	36 (5.0)	12	2	22	33.30
IHC negative	2+ <10%,1+, 0	559 (77.7)	8	3	548	2
Total		719 (100)	111 (15.4%)	7 (0.9%)	601 (83.6%)	

Conclusions: Raising the cut off level for positivity for Her2 IHC staining results in greater than 95% concordance with FISH positivity. Sensitivity and specificity of the IHC assay are also improved.

115 A Retrospective Study of the Diagnostic Accuracy of FNA in Breast Lesions and Implications for Future Use

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Background: The use of fine needle aspiration (FNA) in the diagnosis of breast lesions has declined in many institutions over the last number of years. The use of core needle biopsy for diagnosing breast lesions has increased, partially because FNA cannot distinguish between in-situ and invasive carcinoma, thus hindering the surgical plan for procurement of sentinel lymph nodes. Also, cytology samples may not be suitable for assessing prognostic markers required to determine a patients' need for neoadjuvant chemotherapy. We sought to evaluate the role of FNA for breast lesions and the annual rate of the procedure at our institution over a five-year period.

Design: A retrospective review of breast FNA samples over a 5-year period (2002-2006) was performed followed by identification of cases with corresponding surgical tissue. FNAs done during this period were mostly from palpable breast lesions without image guidance. Pathology residents and fellows collected an average of 4 FNA samples from each lesion using 23 or 25 gauge needles.

Results: A total of 836 FNAs were performed, 255 (30.5%) had histologic follow up. The number of FNAs obtained was 104 in 2002, 170 in 2003, 198 in 2004, 208 in 2005, and 156 in 2006. Each case was placed into one of four categories: non-diagnostic (78), benign (646), atypical/suspicious (47), or malignant (65). Surgical tissue was available for 32% of non-diagnostic cases, 22.4% of benign cases, 80% of atypical/suspicious cases, and 72% of malignant cases. The overall sensitivity and specificity for FNA was 83 and 91% respectively. The overall positive and negative predictive values were 82 and 92% respectively. There were no false positive cases, indicating a PPV of 100% for a diagnosis of malignancy. The false negative rate was 3.5%. For atypical/suspicious cases, 60% showed histologically significant lesions, including carcinoma, atypical hyperplasia, or atypical papillary lesions. The remaining 40% were diagnosed as benign and included fibrosis, fibroadenoma, calcifications, and sclerosing adenosis.

Conclusions: In diagnosing breast lesions by FNA, the literature reports a range of 65-98% for sensitivity and 82-100% for specificity. At our institution the overall sensitivity and specificity for FNA was 83 and 91% respectively. Although there is a national trend away from FNAs of breast lesion, this has not been the experience at our institution. While FNA may not be ideal in the initial evaluation of suspicious lesions, we argue that FNA for clinically benign lesions and recurrent carcinoma has significant value.

116 HER2, Estrogen Receptor and Progesterone Receptor Status in Primary Breast Carcinomas and Paired Axillary Lymph Node Metastases

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Background: HER2, estrogen receptor (ER) and progesterone receptor (PR) status are important in human breast cancer with prognostic and treatment implications. Fluorescence in situ hybridization (FISH) for detecting HER2 gene amplification and IHC for evaluation of ER and PR expression of the primary tumor are commonly used methods. However, assessment of these markers in metastatic tumors is less well studied but may be more biologically significant. In this study we compared HER-2, ER and PR status between paired primary breast tumors and axillary lymph node metastases.

Design: FISH was performed for the assessment of HER2 status of formalin fixed, paraffin embedded tissue sections from 36 cases of lymph node metastasis from cases with known un-amplified HER2 on the primary tumor. ER and PR IHC analysis was performed on serial tissue sections, the results of which were compared to that of the primary tumors.

Results: One case (1/36) was HER2 amplified in a nodal metastasis but not in the primary tumor (no difference by IHC). Three cases (3/36) were ER negative in LN mets and two cases (2/36) were less strongly positive compared to the positive primary tumors. Seven cases (7/36) were PR negative in LN mets in contrast to the matched positive (5 cases) and weakly positive (2 cases) primary tumors. Two cases (2/36) were PR-positive in LN met while the primary breast tumor was negative.

Conclusions: While most paired primary tumors and nodal metastases showed concordance in ER, PR and HER2 status, significant differences were observed in a minority of cases. These findings suggest the possibility that biomarker studies in metastatic breast tumors is informative and can be used to determine further treatment options for metastatic breast cancer patients.

117 A Comparison of a New Technology Against the Established Protocols for HER2 Gene Assessment Using Tissue Microarrays

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Background: HER2 assessment is necessary to establish eligibility for Herceptin therapy. Fluorescent in situ hybridisation (FISH) has long been held to be the gold standard for this assessment but it is a challenging technique both to perform and to interpret. Brightfield in situ hybridisation systems have more recently been developed and have been compared very favourably against FISH but are quite labour intensive. The release onto the market of a fully automated silver in situ hybridisation (SISH) system makes the assessment of the HER2 gene much more accessible for laboratories wishing to perform their own HER2 analysis. However, until now there has been limited data on the reliability of this system in clinical practice.

Design: We introduced the use of SISH into routine HER2 analysis in early 2007 following a successful pilot study to validate the results against FISH and immunohistochemistry. Subsequent to reports of conflicting results of HER2 assessment we decided to perform a study on 241 breast cancer specimens in a tissue microarray for which there was also FISH data available. The TMA slides were analysed according to the manufacturer's protocol and interpreted independently by two pathologists.

Results: There was 98% (236/241) concordance between FISH and SISH. Of the five discrepant results four of the cases had mixed populations of amplified and non-amplified tumour cells in which FISH assessed both populations whilst SISH assessed only the predominant non-amplified population. Immunohistochemistry using the monoclonal antibody 4b5 recorded a 'HER2 2+' result in each case. One case was poorly fixed and should have been repeated for SISH assessment.

Conclusions: SISH is extremely reliable and robust method for HER2 assessment. The full automation means that large numbers of slides can be handled enabling its use as a frontline HER2 assay. The study highlights the issue of heterogeneity in breast cancer and the need to assess it effectively.

118 WT-1 Expression in Primary Mucinous Carcinomas of the Breast

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Background: Strong WT1 expression in a carcinoma of unknown origin virtually excludes a breast primary. Our previous study on WT1 expression in breast carcinomas has shown WT1 expression in approximately 10% of carcinomas that show mixed micropapillary and mucinous morphology {Mod Pathol. 2007; 20 (supplement 2):38A}. Apart from being a diagnostic marker, WT1 is also a molecular target for cancer immunotherapy and thus its immunohistochemical detection in tumor cells is of clinical importance.

Design: To further explore WT1 expression in breast carcinoma, 33 cases of pure mucinous carcinoma and 31 mixed (i.e. mucinous carcinoma mixed with other morphologic subtypes) invasive breast carcinomas were evaluated for WT1 (clone 6F-H2, Cell Marque, Hot Springs, AR) protein by immunohistochemistry. The study was performed on a tissue microarray (TMA) constructed from the above cases. The TMA had 3-fold redundancy with each core diameter of 0.6mm. Only nuclear staining for WT1 was counted. The intensity and percentage cellular staining was quantified

using "H-score" method where the score ranges from 0-300. A score of 11 or more was considered as positive.

Results:

	WT1 Positive	WT1 Negative	Total
Pure Mucinous CA	21	12	33
Mixed Mucinous CA*	9	22	31
Total	30	34	64

*Carcinomas with mucinous morphology mixed with other subtypes.

For the WT1 positive pure mucinous carcinoma, the H-score ranged from 15-210, with a mean score of 88.6 and a median score of 90. Of the 9 WT1 positive mixed carcinomas, 8/9 have admixture of no special type carcinoma with mucinous carcinoma and only 1/9 had a mixed papillary, micropapillary and mucinous morphology. The WT1 H-score for this group ranged from 20-220, with a mean score of 99.4 and a median score of 80. The non-mucinous component showed similar (majority) or slightly weaker (minority) WT1 staining.

Conclusions: 1. Pure mucinous breast carcinomas demonstrate WT-1 nuclear staining in 64% of cases. 2. Invasive breast carcinoma subtypes admixed with mucinous carcinomas may show WT1 staining in up to 29% of cases. 3. Diffuse strong WT1 immunoreactivity in a carcinoma of unknown primary still favors an ovarian serous carcinoma. However, breast primary is not entirely excluded in a tumor with moderate WT1 staining. 4. WT1 expression in mucinous breast carcinomas provides a molecular target for cancer immunotherapy in these relatively indolent breast tumors.The therapy could be useful for patients that experience late systemic recurrences.

119 Management of Pleomorphic Lobular Carcinoma In Situ

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Background: The appropriate management of pleomorphic lobular carcinoma in situ (PLCIS) is unknown. When present on core needle biopsy, subsequent excision is recommended based on the lesion's high-grade cytology and features overlapping with ductal carcinoma in situ (DCIS). However, definitive treatment recommendations are not uniform. In this series, we describe the outcome of patients with PLCIS at or near a margin in resection specimens and provide rationale for obtaining negative margins.

Design: We identified 19 patients with a diagnosis of PLCIS at or near a margin in resection specimens, all of whom were offered chemoprophylaxis (CP) and/or radiation therapy (XRT). The margin status was subdivided as: PLCIS cells at margin without obvious truncation of lesion; PLCIS ≤1 mm from but not involving margin, PLCIS 1.1-2 mm from margin, and PLCIS at least 2.1 mm from margin. To ensure similar biologic potential between margin status groups, no cases with an invasive component > 1 mm were included. E-cadherin was performed and Ki-67 was evaluated in the lesional tissue at or near the margin.

Results: The patients' age ranged from 35-76 years (mean 61.8), length of follow-up ranged from 7-92 months (mean 43.6). Ten of 19 (53%) received CP, 8/19 (42%) received XRT with 4/19 (21%) receiving both. Mammographic abnormalities were present in all cases, with suspicious calcifications being the most common finding (13/19, 68%). PLCIS was the most significant lesion in 15/19 patients (79%), with microinvasive carcinoma present in 4/19 cases (21%). PLCIS was at the margin in 4/19 cases (21%), ≦ 1 mm from the margin in 6/19 cases (32%), 1.1-2 mm from the margin in 4/19 cases (21%), and was at least 2.1 mm from the margin in 5/19 cases (26%). E-cadherin was negative in all cases. Ki-67 immunoreactivity ranged from 1-30% (mean 7.5%) across all margin groups. Considering the group that received CP only, PLCIS was identified in one patient (10%) with a positive margin, after 19 months. All other patients were disease free at last follow-up.

Conclusions: This is the first and largest series that documents recurrence of PLCIS with respect to margin status and addresses outcomes. Given that the risk of recurrence for DCIS is 1-2% per year, the recurrent disease in this PLCIS group suggests that the risk for recurrence for PLCIS is at least similar for DCIS. Therefore, known methods of local control (surgical excision with negative margins (2 mm) with the addition of XRT) maybe appropriate treatment in these patients.

120 Matrix-Producing Carcinoma of the Breast: Clinicopathologic Features Associated with Locoregional Recurrence and Survival

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Background: Matrix-producing carcinoma is a form of metaplastic carcinoma characterized by overt carcinoma with direct transition to a cartilaginous and/or osseous matrix without an intervening spindle cell zone.

Design: Thirty-two cases from 1989-2007 were available for slide review. Correlations between multiple clinicopathologic features were evaluated using Pearson chi-square analysis, and features associated with patient outcome were evaluated by logistic regression analysis.

Results: Fourteen patients (44%) were <50 years of age. Eight (25%) patients had tumors ≤2 cm, and 6 (19%) had tumors >5 cm. Seven patients (22%) had positive axillary lymph nodes. The matrix component in all cases was cartilaginous or chondromyxoid. The matrix was diffuse in 13 tumors (41%), unifocal in 3 (9%), and multifocal in 16 (50%). Matrix comprised ≤10% of the tumor in 14 cases, >10% but <40% in 9 cases, and ≥40% in 9. The matrix component was high grade in 9 cases (28%) and low grade in 23 (72%), whereas the grade of the carcinomatous component was high in 30 cases (94%), intermediate in 1 (3%), and low in 1 (3%). Fifteen tumors (47%) were nodular, 10 (31%) were multinodular, and 5 (16%) were raggedly infiltrating. Nineteen (59%) had central necrosis and 8 (25%) had lymphovascular invasion (LVSI). Nine tumors (28%) had associated DCIS. Follow-up ranged from 3 to 98 mos (median 27 mos). The cumulative proportion of patients with locoregional recurrence-free (LRF) survival and disease-specific (DS) survival at 5 years was 55% and 53%, respectively. In univariate analysis, stage and LVSI were significantly associated with LRF survival (P<0.001 and P=0.003, respectively). Cases with matrix comprising less than 40% of the viable tumor had significantly decreased DS survival (P=0.029). Additional factors associated with decreased DS survival were LVSI, stage, and patient age (P=0.02, P=0.03, and P=0.007, respectively). In multivariate analysis, only LVSI was independently associated with LRF survival (P=0.01), and only stage and patient age were independently associated with DS survival (P=0.04 and P=0.02, respectively).

Conclusions: Matrix-producing carcinoma of the breast is an aggressive subtype, with 55% LRF survival and 53% DS survival at 5 years. Although <40% matrix correlates with decreased DS survival, only patient age, stage and LVSI are independent predictors of worse clinical outcome.

121 A Novel Discriminant Score Based on MIB-1 and HER-2 Immunohistochemistry as an Alternative to Oncotype-DX Recurrence Score in Breast Carcinoma

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Background: Based on the expression of 16 cancer related and 5 reference genes, the Oncotype-DX® assay (OnDXa) calculates a 10-year risk recurrence score (OnRS) for invasive mammary carcinoma (IMC). Results can be stratified into low, intermediate and high risk (LR, IR and HR) groups. In combination with clinical and pathologic parameters, this assay is being increasingly used by oncologists in decisions on adjuvant chemotherapy. Although validated, this assay is expensive, so we sought to determine whether histopathologic parameters together with receptor status and HER-2 in combination with immunohistochemistry (IHC) based prognostic markers (MIB-1 and p53) could be equally predictive in this regard.

Design: Thirty-one cases of IMC (26 ductal and 5 lobular) from 30 patients that were previously assessed by the OnDXa (Genomic Health, CA) were included in the study. Two pathologists graded the tumors for tubule formation, nuclear grade and mitotic count (MBR grade components). IHC of MIB-1 (Dako) and p53 (D07, Dako) was performed and scored using the Apero ScanScope automatic slide scanner and results quantified using the ImagePro Plus 6 software (MediaCybernetics, MA). ER, PR and HER-2 status was also analyzed.

Results: The median IMC size was 1.3 cm (range 0.3-4.5). Ten of 31 tumors were LR, 18/31 IR and 3/31 HR by OnDXa. In the LR tumors MBR was grade I in 5/10 and grade II in 5/10; in the IR group MBR was grade I in 5/18, grade II in 8/18 and grade III in 5/18; and in all 3 HR tumors the MBR was grade II. A positive correlation was found between OnRS and mitotic index (R_s=0.5, p=0.004) and the MIB-1 score (R_s=0.49, p=0.006), and a significant association with HER-2 for all IMC cases. A multivariate regression model was used to create a discriminant score (DS) to predict HR cases, as follows: DS = - 7.78 + (0.17 x MIB-1) + (13.60 x HER-2). Using ROC analysis, a best DS cutoff (-0.966) was found that correctly classified 98% of the HR tumors (sensitivity = 100%, specificity = 95.5%). The correlation between the DS and the Oncotype-DX® score was highly significant (R=0.76, p<0.0001).

Conclusions: A discriminant score (DS) based on MIB-1 and HER-2 IHC was obtained from the data that is predictive of HR Oncotype-DX® score. This novel DS is an inexpensive alternative to OnDXa that can potentially be used to select patients for adjuvant chemotherapy.

122 UPARAP/Endo180 Expression in Invasive Breast Carcinoma and Its Relation to Patient Outcome

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Background: Local growth, invasion, and metastasis of malignancies of invasive breast carcinoma involve extensive degradation and remodelling of the surrounding, collagen-rich connective tissue. Urokinase plasminogen activator receptor-associated protein (uPARAP)/Endo180 is an endocytic receptor recently shown to play a critical role in the uptake and intracellular degradation of collagen by mesenchymal cells. However, the expression of this protein and its clinical significance in breast cancer is unknown.

Design: immunohistochemistry was used to investigate the expression of (uPARAP)/Endo180 in tissue microarrays of a large (n=880) well-characterized series of human breast carcinomas using blinded semiquantitative scoring, in addition to a set of well known biological markers in breast cancer.

Results: (uPARAP)/ was expressed in (5.7%) of invasive breast cancer, and in (78.8%) in the stroma surrounding these tumours. Positive expression of Endo 180 in the tumour cells was significantly correlated with negative steroid receptor as, ER (P=.013), and AR (P=.001), negative luminal cytokeratines like CK7/8(P= .041). Further more, a positive correlation was found between Endo180 expression and basal subtype of breast carcinoma (P= .003). In addition to the association between its expression and shorter disease free interval (P=.01).

Conclusions: The association between (uPARAP)/Endo180 expression in malignant cancer cells with basal phenotype and its association with poor patient outcome could explain the aggressive behaviour of these types of tumour.

Cox proportional hazards analysis for predictors of Disease free survival effects of tumour grade, size, lymph node stage, HER2, ER, and end180 expression status in invasive breast cancer

Variables	Hazard ratio	95%Confidence interval	P- value
Grade*	1.363	.98-1.88	.059
Tumour size ≥1.5cm**	2.020	1.20-3.40	.008
Lymph Node Stage (positive) ***	1.814	1.42-2.31	<.001
Endo-180 positive expression	1.983	1.02-3.85	.044
HER2 expression****	1.244	1.06-1.46	.008
ER positive status	.879	.57-1.35	.577

* fitted as linear term, i.e. increase in risk for change in grade of one unit

123 Needle Core Biopsy of Male Breast Avoids Surgical Excision in Appropriately Selected Clinicopathological Situations

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Background: Needle core biopsy (NCB) is now considered a routine and reliable diagnostic procedure in the *female* breast. Thus far, there is only limited published experience regarding the utility of NCB in the *male* breast.

Design: All NCB obtained from male breasts were retrieved from departmental archives. Related clinical, and follow-up, data were obtained.

Results: Pathological material (and related clinical data) from thirty-one (31) NCB from male breasts (1999-2007) were reviewed. All patients underwent unilateral NCB (right:16, left 15). Mean age of patients was 57 (range: 18-88). 28 patients presented with a mass, 2 with nipple discharge and 1 with "redness of breast". Mean number of needle cores obtained during the biopsy procedure was 2.8 (range 1-9). Histopathological findings included gynecomastia (14 cases), invasive carcinoma (3), myofibroblastoma (2), intraductal carcinoma (1), lymphoma (1), angiolipoma (1), hemangioma (1), atypical "lactational-like" hyperplasia (1) and fat necrosis (1). Inactive and unremarkable breast tissue was seen in 4 cases, and fibroadipose tissue (without breast glandular tissue) in 2 cases. Subsequent excisional biopsies (4 cases) and mastectomies (2) showed no discordant results- including in all cases of carcinoma (4), hemangioma (1) and atypical "lacataional like hyperplasia" (1). The latter case (which had presented with nipple discharge) was subsequently diagnosed with a pituitary prolactinoma. Fine needle aspiration biopsy had been performed in 6 cases with the following results- positive: 1 case (of invasive carcinoma), atypical: 2 (both of gynecomastia), gynecomastia: 1, non-diagnostic (sparsely cellular): 2. Follow-up showed no evidence of any missed diagnosis in NCB cases whether or not surgical excision was subsequently performed.

Conclusions: Needle core biopsies can reliably sample a wide spectrum of benign and malignant pathological entities that afflict the male breast. This minimally-invasive procedure could avoid surgical excision in appropriately selected clinicopathological situations.

124 Periostin (POSTN, OSF2) Expression in Stroma of Normal Breast Tissue and Pre-Invasive Breast Lesions

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Background: The importance of interactions between stromal cells and epithelial cells in established breast cancers is undisputed. However, the role of stromal-epithelial interactions in earlier stages of breast tumor progression is less well-understood. Periostin (POSTN, OSF-2) is a secreted protein that plays a role in cell adhesion. We recently showed that a subset of invasive breast carcinomas is associated with a stromal response that involves upregulation of POSTN and that in these cases POSTN is expressed in the cancer-associated fibroblasts. The purpose of this study was to evaluate POSTN mRNA expression by in situ hybridization in normal breast tissue and pre-invasive breast lesions to assess its potential role in earlier stages of breast tumorigenesis.

Design: The level of gene expression for *POSTN* was assessed using RNA in situ hybridization (ISH) on a tissue microarray that contained 40 normal breast tissues, 12 radial scars, 20 non-proliferative lesions (NP), 20 sclerosing adenosis (SA), 20 usual ductal hyperplasias (UDH), 17 atypical ductal hyperplasias (ADH), 15 low-intermediate grade DCIS, 18 high grade DCIS, and 18 invasive ductal carcinomas.

Results: POSTN mRNA was expressed at very low levels in stroma surrounding normal breast tissue, NP, and radial scars. In contrast, upregulation of POSTN expression was seen in the stroma of 17/18 (94%) invasive ductal carcinomas. Among pre-invasive lesions, POSTN upregulation was most common in stroma surrounding high grade DCIS (10/18 cases; 56%). A small number of low-intermediate grade DCIS (2/15 cases; 13%), ADH (1/17 cases; 6%), UDH (4/20 cases; 20%) and sclerosing adenosis (1/20 cases; 5%) also showed upregulation of POSTN in the surrounding stroma.

Conclusions: Expression of POSTN in high grade DCIS and other pre-invasive breast lesions suggests new heterogeneity in these lesions and raises the possibility that POSTN may have a role in breast tumor progression. Future studies of patients with pre-invasive lesions and known outcome will address whether stromal expression of POSTN in these lesions is associated with an increased risk for development of breast cancer.

125 The Expression of Cytokeratin 5/6 in Invasive Lobular Carcinoma of the Breast: Evidence of a "Basal-Like" Subset

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Background: Analysis of gene expression profiling data on breast cancers has revealed molecular subclasses that may have prognostic significance. The basal-like breast cancers, one of these "molecular subclasses", have been associated with a significantly worse overall and disease-free survival as compared with most of the other subclasses. Previous studies on basal-like cancers have been performed predominantly on the ductal histotype. This study was designed to evaluate the significance of the expression of Cytokeratin (CK) 5/6, a commonly used surrogate marker for the basal-like phenotype, in invasive lobular carcinomas (ILC).

Design: Clinical and pathologic data, including data on the the immunohistochemical expression of CK5/6, ER, PR, HER2/neu and E-Cadherin were collected in a group of 82 consecutive, archived ILC diagnosed in 82 women [age range 29-73 years (mean 51.9)].

Results: All cases were E-Cadherin negative. CK 5/6 was positive in 14 (17%) of 82 cases and was entirely negative in the remaining 68 cases (83%). In 8 out of the 14 CK5/6[+] cases, staining was diffuse and intense. In the remaining 6 cases, staining was patchy (>1 low power field between positive areas) but still of high intensity. CK5/6[+] cases were significantly more likely than CK5/6[-] cases to be ER[-] (43% versus 0% respectively, p<0.0001). CK5/6[+] cases were also significantly more frequently of modified Scarff Bloom Richardson (MSBR) histologic grade 3, as 7 (50%) of the 14 CK5/6[+] cases were of histologic grade 3, as compared with only 6 (8.8%) of 68 of the CK5/6[-] cases (p=0.0009). Notably, the average mitotic index in the CK5/6[+] group was 11/10 high power fields, as compared with 7/10 high power fields in the CK5/6[-] group (p=0.07). Overall, there were no distinct morphologic differences between the 2 groups, and both displayed the well-characterized cytoarchitectural features of ILC. CK5/6[+] and CK5/6[-] cases did not significantly differ with respect to patient age, frequency of PR expression, tumor size, rate of axillary node involvement or HER2/neu overexpression.

Conclusions: It is concluded that 17% of ILC express CK5/6, and that CK5/6[+] cases are more likely to be ER[-] and have a high MSBR histologic grade. Since these findings are characteristic of ductal basal-like breast cancers, our results suggest that there is a basal-like subset for ILC with potentially distinct clinicopathologic features. Future studies are required to define the prognostic significance of CK5/6 expression in ILC.

126 Assessment of 1183 Screen-Detected, "Probably Benign" Circumscribed Masses by Cytology and Core Biopsy with Long Term Follow Up Data

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Background: Discrete masses are commonly detected during mammographic screening. Most such lesions are benign. For lesions without pathognomonically benign imaging features but still regarded likely to be non-malignant (Tabar grade 3), reliable biopsy results would be a clinically useful alternative to mammographic surveillance.

Design: Between Jan 1996 - Dec 2005 discrete masses detected in a large, population based, breast cancer screening program are included. Patient demographics, FNA, core and surgical biopsy results are tabulated. The pathology of excised lesions was sourced. Information regarding interval cancers was obtained from the State Cancer Registry records and through follow-up of clients in subsequent rounds of screening.

Results: 1183 lesions, mean diam. 13.3 mm (+/- 8.3mm), mean age 55.1 yrs (+/- 8.8yrs) are included. After diagnostic work up 98 lesions (8.3%) were malignant, 1083 were non-malignant and a final histologic diagnosis was not established in 2 lesions. In the 27 months after assessment, no interval cancers were attributable to these lesions and during a mean follow up of 54.5mths, available in 84.9% of eligible women, only 1 cancer has developed in the same quadrant as the original lesion, although the two processes are believed to be unrelated. FNAB performed in 1149 cases was definitive in 80.5% cases (882 benign, 43 malignant) with a NPV of 99.8% (880 of 882) and a PPV of 95.2% (40 of 42, both intraductal papillomas). Core biopsy was performed in 178 lesions, mostly for indefinite cytology. Core biopsy was definitive in 79.8% cases (57% benign 22% malignant) with a PPV of 100% and NPV of 99.0%.

Conclusions: In experienced hands FNAB is an accurate first line diagnostic modality for the assessment of probably benign screen-detected discrete masses, providing definitive results in 80.5% of cases. When used as a second line modality, core biopsy had a similarly high rate of definitive diagnosis at 79.8%. The stepwise approach to the use of FNA and core biopsy would reduce substantially the proportion of cases requiring surgical diagnostic biopsy. Given the low probability of malignancy and the imperative to limit the morbidity associated with cancer screening, the demonstration of the reliability of FNAB as a minimally invasive but highly accurate test for this particular subset of screen-detected lesions has significant clinical utility.

127 HER2 Testing: IHC, CISH and RT-PCR Correlation Study

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Background: By testing HER2 over-expression, physicians can accurately select patients likely to benefit from target therapies. Most laboratories use immunohistochemistry (IHC) initially. In cases with doubts and 2+ results the gold standard method is fluorescent in situ hybridization (FISH). Alternatively, cromogenic in situ hybridization (CISH) detects amplification using a peroxidase reaction, viewed by a standard light microscope. Real-time PCR (RT-PCR) an optimal quantitative assay has been also recently used. HER2 testing has a key role in breast cancer management, so the aim of the study was to compare IHC standardized procedure against CISH and RT-PCR, evaluating correlation and minimizing false positive fraction from IHC technique.

Design: We analyzed 52 primary breast cancer samples, fixed in 10% formalin and embedded in paraffin. IHC was performed using policlonal antibody anti Her 2 (DAKO), microwave antigenic recovery, detection system EnVision (Dako) and developed with diaminobenzidine. Results were interpreted as ASCO guidelines.CISH essays were run following Zymed protocol: CISH results were evaluated using light microscope at low and high power magnification. HER2 signals per nucleus were counted: <6 copies non amplification and > 6: positive amplification. For RT- PCR, DNA was extracted from 4-6 slices of the same paraffin blocks and studied in the LightCycler using Her2 DNA quantification kit (Roche), based on the simultaneous quantification of Her2 DNA copies relative to a reference gene and normalized to a calibrator. CISH and RT-PCR assays were done blindly to the IHC results.

Results: Table 1: HER 2 status: IHC, CISH and RT-PCR correspondence.

HER 2 status: IHC, CISH and RT-PCR correspondence

IHC	CISH Non Amplified	CISH Amplified	CISH NonReactive	RT-PCR Non Amplified	RT-PCR Amplified	RT-PCR NonReactive
0	9	0	0	9	0	0
1+	13	0	0	10	1	2
2+	1	5	4	2	8	0
3+	0	20	0	1	16	3

Conclusions: There was lofty correlation among IHC, CISH and RT-PCR results in reactive tissues. High percentage of non reactive samples was found, due to fixation and processing issues. In our experience, CISH and RT-PCR are specific, sensitive, and easily applicable methods for detection of Her-2/*neu* gene amplification. CISH and RT-PCR are a promising, practical alternative to FISH in adequate processed samples.

128 Clinicopathologic Features of DCIS in Women under Age of 35

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Background: DCIS is rare under age of 35 years. It has been suggested that DCIS in younger women is associated with higher recurrence rate and worse outcome. We performed a retrospective review of the clinicopathologic characteristics of DCIS in women ≤35 years old.

Design: We identified 21 women ≤35 years presenting with DCIS diagnosis from 2005-2007. Only cases with pure or extensive DCIS with a minor invasive component in final surgical specimen were included. We reviewed clinicopathologic data as well as the immunohistochemistry (IHC) profile including ER, PR, HER-2, p53, Ki67, and CK5/6. DCIS was graded as recommended by CAP.

Results: Mean age was 32 years (range 26-35 years). Twelve patients were asymptomatic (57%) and nine presented with pathologic nipple discharge or palpable finding (43%). Strong family history of breast carcinoma was known in 8/21 (38%). Five cases (24%) were identified during lactation or pregnancy. Twelve (57%) required mastectomy. DCIS size was >5cm in 43%. DCIS showed prominent involvement of lobules in 74% of cases. Nuclear grade of DCIS was 3 in 71%. High nuclear grade was significantly more common in our group as compared to a group of 278 consecutive DCIS cases from the past 6 months (Chi square analysis p=0.037). DCIS was ER+ in 76%, HER-2 positive in 32%, p53 positive in 30%. The mean Ki67 score was 21% (range=5-47%) and was 10% or higher in 66% of cases. Cases were classified based on IHC as luminal A (63%), luminal B (10%), HER-2+ (21%) and triple negative (5%). Invasive carcinoma was associated with DCIS in a surgical specimen in 12/21 cases (representing a minor component in all cases). Mean size of invasive carcinoma was 0.5 cm (range <0.1-2.0cm). Positive lymph nodes were found in 4 patients.

Conclusions: In contrast to older women, those ≤35 years old frequently present with symptomatic DCIS (43% in our population). They are often diagnosed during lactation or pregnancy (24% of cases) and have a strong family history of breast cancer (38%). DCIS in young women is typically extensive (>5cm), involves lobules, and is associated with high proliferation index. High nuclear grade of DCIS is significantly associated with DCIS at young age. The frequency of luminal, HER-2 positive and basal-like subtypes is similar as that reported for population-based DCIS studies. It appears that the aggressiveness of DCIS in young women is related to high nuclear grade and not to an increased frequency of an aggressive molecular subtype.

129 Atypical Ductal Hyperplasia in Core Biopsy: Are There Indictators of Upgrade at Excision?

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Background: Atypical ductal hyperplasia (ADH) in core biopsy (CB) warrants subsequent surgical excision (SE). This is based on previous reports showing that ADH in CB is associated with an upgrade to malignancy (DCIS or invasion) in 10 to >50%. It has been suggested previously that the quantification of size or the degree of atypia may be used to stratify ADH lesions in CB to those that need SE and those that may be adequately managed by follow-up. We studied ADH lesions diagnosed in CB to identify potential predictive factors associated with upgrade.

Design: We reviewed 81 consecutive cases of ADH diagnosed in CB specimens 01/06-07/07, negative for DCIS or invasive carcinoma. ADH was characterized according to architectural pattern, number and size of foci, and presence of severe atypia (bordering DCIS). Radiologic information was retrieved from the charts.

Results: Of 81 retrieved cases 18 had no follow-up surgery, one diagnosis was converted to DCIS and 17 were excluded due to a history of ipsilateral invasive carcinoma or DCIS. The mean age of remaining 45 patients was 52 (range 35-80). The mean time period between CB and SE was 4.2 weeks. BIRAD score was available in 32 cases (4A-4, 4B-24, 4C-4). Six cases were biopsied for mass lesion, 39 for calcifications. Biopsies were performed using stereotactic (40), US (3), or MRI (2) guidance. ADH foci in CB varied in size (1 - 3 mm) and number of foci (1-5). Malignancy was identified in SE in 8 (17%) cases: 2 IDC, 1 ILC, and 5 DCIS. Two IDC cases (7 mm and 8 mm) were both associated with radiological mass lesion. ILC was microinvasive (1 mm) and in this case ADH was associated with high grade LCIS in CB. One DCIS was grade 2, others were grade 1, with the mean size 5.1 mm (1.5-15mm). None of the BIRAD-4A cases were upgraded. Cases with severe atypia in CB and cases with history of contralateral carcinoma were more likely to be associated with upgrade (chi square $p<0.05$). There was no association between upgrade and architectural pattern, size, or number of ADH foci.

Conclusions: In our study 17% of ADH in CB were upgraded to malignancy in SE. Although the upgrade was associated with radiologic mass lesion, history of contralateral carcinoma and severe atypia (bordering DCIS), no combination of these factors was useful in predicting which cases would be upgraded. Upgrade to invasive carcinoma was limited to cases with radiologic mass finding. Quantification of atypia in CB is not helpful in identifying ADH lesions that need excision versus those that could be managed by follow-up.

130 Adenoid Cystic Carcinomas of the Breast Express ER-α36, a Novel Variant of Human Estrogen Receptor-α (ER-α66)

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Background: Adenoid cystic carcinoma (ACC) of the breast is considered a special type of breast carcinoma with favorable prognosis, that does not express classic estrogen receptor alpha (ER-α66), progesterone receptor (PR) and HER-2 ("triple negative carcinoma"). We recently identified and cloned a novel variant of ER-α66, named ER-α36, which mediates membrane initiated steroid signaling (MISS). MISS is dependent on ER interaction with receptor tyrosine kinases, and we investigated expression of ER-α36 and epidermal growth factor receptor (EGFR) in breast ACC.

Design: Fourteen patients with breast ACC with long follow-up (up to 27 years) were studied for expression of ER-α66, PR, HER-2, EGFR and ER-α36 using immunohistochemical methods. ER-α36-specific antibody was custom-made by the Alpha Diagnostic International (San Antonio, TX) against the unique 20 amino acids at the C-terminal of the ER-α36, other antibodies were from commercially available sources. Mammary carcinoma cell line (MDA-MB-231) that lacks expression of ER-α66, PR and HER2, but expresses ER-α36 and EGFR, was used as a positive control.

Results: No lymph node metastasis (pN0) was seen in any of the ACC cases at the time of the surgery. However, five of 14 patients developed metastatic disease (between 6 and 23 years after the surgery), and two died of disease (7 and 27 years after the surgery). No tumor showed ER-α66, PR or HER2 expression, but 8/11 cases showed ER-α36 expression in membranous and cytoplasmic distribution. EGFR was expressed in 8/14 cases. In MDA-MB-231 cells (an experimental model of triple negative mammary carcinoma/ER-α36+/EGFR+), 17β-estradiol (E2) strongly induced rapid phosphorylation of the MAPK/ERK1/2, but had only a weak effect in ER-α36 siRNA knock-down MDA-MB-231 cells.

Conclusions: Despite earlier claims that ACC rarely metastasize, this tumor type is a typical example of a "triple negative" breast carcinoma with significant mortality upon long follow-up. Although consistently negative for ER-α66 expression, ACC expresses ER-α36, and it is this receptor variant that shows strong E2 signal transduction and an increased cell growth in "triple negative" carcinomas. This non-genomic estrogen signaling probably involves membranous interaction between EGFR and ER-α36, resulting in activation of mitogen activated protein kinase pathway.

131 Comparison of the Clinicopathologic Features and Prognosis of Epithelial and Mixed Metaplastic Carcinomas of the Breast

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Background: Metaplastic carcinomas of the breast (MCB) are classified into those that are purely epithelial and those with mixed epithelial and mesenchymal components. To the best of our knowledge, the pathologic features, prognostic marker profile, and natural history of these two subtypes have not been compared previously. We thus sought to determine if there are any differences between the clinicopathologic features and prognosis of these neoplasms.

Design: A computerized search of the surgical pathology database at our institution was performed for the years 1994 to 2007. Cases that came up using the key words "metaplastic carcinoma" and "breast" were reviewed, and pertinent data, including patient demographics, surgical pathology and prognostic marker reports, and therapies received were analyzed. Information on clinical outcome was obtained using the latest patient encounter summaries.

Results: Fifteen epithelial and eleven mixed MCB cases were retrieved. The mean tumor size was 3.7 cm (range 1.1-10 cm) for the epithelial group and 2.5 cm (range 1.5-6 cm) for the mixed group. Nodal metastasis was observed in 40% of epithelial cases and none in the mixed group (p=0.035). Estrogen receptor (ER), progesterone receptor (PR), and HER2/neu were each negative in 90% of epithelial cases. On the other hand, ER was negative in 100%, PR in 71%, and HER2/neu in 86% of mixed cases. A high proliferation rate of tumor cells (>16% of tumor nuclei stained with Ki67) was noted in 90% of epithelial and 100% of mixed cases, whereas p53 was positive in 71% of epithelial and 75% of mixed cases. Four (27%) epithelial carcinomas had an aggressive course, two of which metastasized to the brain, one to the lung, and one that recurred several times and metastasized widely, leading to death. Only one (9%) mixed case followed an aggressive course with metastases to the femur and uterus. The epithelial neoplasms appeared to have a higher rate of distant metastasis than the mixed tumors (p=0.3). The remaining epithelial cases were disease-free after a mean follow-up period of 2.7 years while the other mixed tumors were disease-free after a mean follow-up period of 4.3 years.

Conclusions: Our study shows that epithelial MCB are more likely to metastasize to lymph nodes than mixed MCB. Moreover, epithelial MCB appear to have a higher propensity to metastasize to distant sites, although this difference is not statistically significant. A larger case series is needed to better characterize the clinicopathologic features and prognosis of these MCB subtypes.

132 Comparative Immunohistochemical Analysis of EGFR, CD117, and CD10 Expression in Benign and Malignant Phyllodes Tumors of the Breast: A Diagnostic and Prognostic Utility Study

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Background: Phyllodes tumor is a rare biphasic neoplasm in the breast. Although recent studies have suggested a role for EGFR in phyllodes tumor progression and for CD117 in predicting recurrent behavior, these associations have not been clearly elucidated. Similarly, although CD10 stromal expression has been reported in mammary fibroepithelial lesions, its significance in phyllodes tumor biology has not been studied. To address these questions, we undertook a study to compare the expression of EGFR,

CD117, and CD10 between benign and malignant phyllodes tumors of the breast.
Design: Representative areas from five benign and five high grade malignant phyllodes tumors were selected for construction of tissue microarrays and then stained using antibodies against EGFR, CD117, and CD10. The intensity and extent of staining of these markers in these neoplasms were analyzed.
Results: EGFR was negative in both stromal and epithelial elements of all benign tumors (0/5), whereas all malignant cases (5/5) exhibited diffuse moderate to strong stromal staining (p=0.008). CD117 was consistently negative in the stromal components but positive in the epithelium of all benign and malignant cases. Stromal CD10 expression was observed in all benign cases and in only two malignant cases. CD10 was also consistently positive in the myoepithelial cells of all benign and malignant cases, the staining intensity being greater than that in the stroma, indicating that CD10 may serve as a reliable breast myoepithelial marker. Prognostic markers were obtained in two of five malignant cases and showed similar results, with negative expression of estrogen receptor, progesterone receptor, and Her-2/neu; overexpression of p53; and high Ki67 expression (>16%).
Conclusions: Our study indicates that EGFR expression may be used in differentiating benign from malignant phyllodes tumors. Moreover, EGFR overexpression in malignant but not in benign phyllodes tumors suggests a role for this marker in tumor progression, and that anti-EGFR agents may be potentially useful in the treatment of these neoplasms. CD117, on the other hand, appears to have no diagnostic or prognostic value. CD10 nonspecifically stains benign and malignant phyllodes tumors and therefore appears to have no role in tumor progression. We are analyzing additional cases to provide more conclusive evidence on the diagnostic utility and prognostic value of these markers in these neoplasms.

133 Amplification and Overexpression of MAP3K3/MEKK3 in Human Breast Cancer

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Background: The long arm of chromosome 17 is frequently abnormal in breast cancer, usually seen as 17q gain. Several genes on 17q (ERBB2, BRCA1 and PPM1D) are important in tumorigenesis of breast cancer. However, the nature of anomalies in other regions, including 17q21, 17q22-q24 and 17q25, remains unclear. A member of the mitogen activated protein kinase kinase kinase (MAP3K) family of signaling proteins, known as *MAP3K3*/MEKK3, is mapped to 17q23.3. MEKK3 has been shown involving in tumorigenesis in several different cancers. We explored the expression of MEKK3 in breast cancer cell lines and in human breast cancer tissue.
Design: We performed quantitative reverse-transcriptase PCR (RT-PCR), genomic quantitative PCR and fluorescence in situ hybridization (FISH) to examine the levels of *MEKK3* gene. Sixteen cases of invasive ductal carcinoma were tested. For quantitative RT-PCR, 100 ng of total RNA was used. RT-PCR was performed with ABI PRISM 7900 Sequence Detection System and the data were analyzed with SDS software v2.0 (Applied Biosystems). The results of RT-PCR are expressed as Ct value. The mRNA levels of MEKK3 were normalized against GAPDH. For genomic quantitative PCR, 100 ng of genomic DNA extracted using genomic DNA extraction kit (Qiagen) was used. Each target gene was measured in duplicate. For FISH analysis, the chromosome 17 centromere (green) and BAC RP11-51F16 DNA covering the *MEKK3* gene locus (red) were labeled with FITC and dUTP, respectively.
Results: MEKK3 is highly overexpressed in MCF-7 (the breast cell line tested), compared to MCF-10A (a non-tumorigenic mammary epithelial cell line). The FISH analysis showed that The *MEKK3* gene is amplified in MCF-7 with 10 copies, comparing to the control, MDA-MB435 (a breast cancer cell line known to have normal 17q status). By genomic quantitative PCR, the MEKK3 copy number gain was observed in three among sixteen breast tumors. FISH analysis was performed in these three breast cancer specimens and confirmed the MEKK3 copy number gain. Three to four copies of MEKK3 loci were detected in the majority of tumor cells from these three specimens.
Conclusions: Amplifcation and overexpression of *MEKK3* gene was found in both breast cancer cell line and 18% of breast tumor specimen. Overexpression of *MEKK3* gene may play a critical role in breast tumorigenesis and serve as a potential prognostic marker/ therapeutic target in a subpopulation of breast cancers.

134 Aurora Kinases A and B Overexpression in Breast Cancer: Biological Significance and Clinical Implications

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Background: Overexpression of Aurora A (AA) and Aurora B (AB) kinases can induce centrosome amplification, aneuploidy and cellular transformation. The aim of this study was to detect AA and AB kinase expression in invasive breast cancers and to test the antiproliferative effects of Aurora kinase inhibitors in breast cancer cell lines.
Design: Archival invasive breast cancer tissue specimens from 35 patients were immunostained for AA and AB kinases expression. Normal breast tissue from mammoplasty specimen was used as controls. AA and AB kinases expression were assessed on a scale of 1 to 3 (1+ - weak, 2+ - intermediate, 3+ - strong). Samples with either 2+ or 3+ staining were considered positive for AA and AB kinases overexpression. To test the antiproliferative effect of Aurora kinase inhibitor, we used 2 breast carcinoma cell lines (MCF7 and MB231). The cell were incubated with varying concentrations of Aurora kinase inhibitor VE-465 for 24 to 48 hours and tested by MTS proliferation assay and DNA/PI staining.
Results: Aurora kinase A was overexpressed in 28/35 (80%) and Aurora kinase B in 27/35(79%) invasive breast carcinomas. None or weak staining was observed in normal breast tissue. In the inhibition assay with Aurora kinase inhibitor VE-465, MB231 cell lines showed a significant inhibition at 24 to 48 hours of incubation. The MCF7 cell lines demonstrated inhibition only at 48 hours of incubation. DNA ploidy analysis by flow cytometry revealed that the inhibition is primarily due to G2 cell cycle arrest in both MCF7 and MB231 cell lines.
Conclusions: This analysis showed AA and AB kinases overexpression in 80% of invasive breast carcinomas. No overexpression was observed in normal tissue. These findings suggest that overexpression of AA and AB kinases may be involved in the pathogenesis of human breast carcinomas. The antiproliferative effect of Aurora kinase inhibitor in breast cancer cell lines further emphasizes the therapeutic potential of inhibiting this family of kinases in breast cancer.

135 Histopathological Characteristics of Primary Tumor in Breast Cancer Patients with Isolated Tumor Cells in Sentinel Nodes

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Background: Adjuvant treatment decisions in patients with isolated tumor cells (ITCs) in the sentinel node (SN) remain controversial. The aim of this study was to determine if there is difference in the primary tumor characteristics (PTC) between tumors with ITCs and those with no nodal metastases (N0) or those with micro- or macrometastases (MM).
Design: This was a retropsective study of all sentinel lymphadenectomy procedures performed betwen 2001-06. Students two tailed t-test and one way ANOVA analyses was used to determine significance.
Results: 552 patients underwent sentinel lymphadenectomy; 197 (36%) had a positive SN, 355 (64%) were SN negative. Of 197 cases, 35 (18%) were classified as ITCs and 162 (82%) as MMs. The mean age of the positive SN group was 56 (27-92) years and the mean tumor size was 2.3 cm(0.1-11). Primary tumor characteristics of the N0, ITC and MM groups are listed in Table 1. The ITC group had significantly more lymphovascular invasion (LVI) and a higher proliferative rate than the N0 group ($p<0.05$). In comparison to the MM group, the ITC group had significantly less LVI, a lower proliferative rate and smaller tumor size ($p<0.05$). There was no significant difference in the age, ER, PR or Her 2 neu status, histologic type or grade in ITC versus N0 or MM groups.
Conclusions: Proliferation and LVI of the primary tumor are significantly different between the ITC, N0 and MM groups suggesting that ITCs may have different biology than the N0 or MM groups. Additional studies are needed to determine if these factors should be used when recommending adjuvant therapy for patients with ITCs in their SN.

Table 1: Primary tumor characteristics of N0, ITC and MM groups

	N0	ITC	MM
Age	61 (24-94)	58 (31-87)	56 (27-92)
Tumor size	1.5 (0-7)	1.6 (0-4)	2.4 (0-11)
ER positive	259 (83%)	29 (88%)	131 (85%)
Her 2 neu positive	19 (7%)	4 (13%)	19 (13%)
Grade	1-63 (18%), 2 - 212 (60%), 3 - 76 (22%)	1- 2 (6%), 2 - 24 (68%), 3 - 9 (26%)	1- 17 (11%), 2-107 (66%), 3 - 37 (23%)
LVI present	16 (5%)	6 (18%)	63 (40%)
Ki67	Favorable 83 (36%) Borderline 65 (28%) Unfavorable 82 (36%)	Favorable 4 (16%) Borderline 14 (56%) Unfavorable 7 (28%)	Favorable 33 (28%) Borderline 32 (28%) Unfavorable 51 (44%)
Histology type	IDC 230 (65%) Non ductal 75 (21%) DCIS 49 (14%)	IDC 26 (74%) Non ductal 5 (14%) DCIS 4 (12%)	IDC 104 (64%) Non ductal 44 (27%) DCIS 14 (9%)

136 Impact of the New American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) Guidelines on the Determination of Breast Cancer HER2 Status

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Background: In January 2007, ASCO/CAP issued new guidelines for HER2 testing of breast cancers (J Clin Oncol 2007;25:118-145). Among the changes in these guidelines, a breast cancer must now show uniform, intense membrane staining in >30% of invasive tumor cells (rather than >10%) to be considered HER2 positive (3+) by immunohistochemistry (IHC). The stated goal of this change is "to decrease the incidence of false positive 3+". However, its impact on the assignment of HER2 status by IHC and on correlation between IHC and fluorescence in situ hybridization (FISH) results has not been previously evaluated.
Design: Between January and August 2007, both IHC (Dako rabbit polyclonal antibody A085) and FISH (PathVysion, Vysis) were performed on 215 consecutive invasive breast cancers accessioned at our institution. IHC results were scored as 0-1+ (negative), 2+ (equivocal) and 3+ (positive) using both the original HercepTest criteria and the new ASCO/CAP guidelines. Results of IHC were compared with those obtained by FISH. Based on the new guidelines, FISH results were scored as not amplified, equivocal or amplified when the HER2/CEP17 ratio was <1.8, 1.8-2.2, or >2.2, respectively.
Results: Using the original HercepTest scoring criteria with a 10% threshold for 3+ positivity, 153 cases (71%) were IHC 0-1+, 35 (16%) were 2+, and 27 (12%) were 3+. Correlation between IHC and FISH results is shown in the Table. Ninety-five percent of cases that were IHC negative also lacked gene amplification by FISH. Conversely, 93% of IHC 3+ cases showed HER2 gene amplification. Of note, all 27 cases scored as 3+ by IHC using the >10% cut-off remained 3+ using the new cut-off of >30%. Thus, the concordance between IHC 3+ cases and FISH results was unchanged when the new ASCO/CAP guidelines for IHC scoring were employed.
Conclusions: The results of this study suggest that the new ASCO/CAP guidelines for HER2 scoring are unlikely to have a substantial impact on the proportion of cases

categorized as HER2 positive (3+) by IHC or, in turn, on the level of concordance between IHC and FISH among IHC-positive cases.

Comparison of HER2 by IHC and FISH

	FISH Not Amplified (HER2/CEP17 <1.8)	FISH Equivocal (HER2/CEP17 1.8-2.2)	FISH Amplified (HER2/CEP17 >2.2)
IHC 0-1+ (negative)	145	2	6
IHC 2+ (equivocal)	22	4	9
IHC 3+ (positive)	2	0	25

137 Monomorphic Epithelial Proliferation (MEPs) Lesions Associated with Ipsilateral Breast Failure (IBF) Carcinomas Following Breast Conserving Therapy (BCT) and Radiation Therapy (RT): Evidence That MEPs Are the Background Pool of Precursor Lesions from Which Carcinomas Arise and Are a Key IBF Risk-Reduction Lesion Eradicated by RT

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Background: It is generally thought that IBFs after BCT-RT are derived from incompletely eradicated CA. We previously suggested MEPs are the pool of partially transformed clones from which breast carcinoma arises and that RT functions to reduce IBF risk primarily by eradicating MEPs. RT produces diffuse marked lobular atrophy, providing a "clean slate" against which MEPs can be more easily studied. We examined 62 IBF salvage mastectomies to examine relationships between MEPs and IBFs.

Design: 51 IBF salvage mastectomies (SMs) with BCT and RT that had initial excision specimens with low risk of substantial residual CA margins [negative (>4.2 mm), near-least amount] were studied (mean 38.66 blocks/ case). Initial CA-IBF CA clonality was determined with a minimum 20 informative marker AI PCR assay. MEPs were a slightly overcrowded, predominantly single layer of monomorphic, luminal epithelial cells that involved TDLUs in an overgrowth extension pattern.

Results: All 51 SMs had RT lobular atrophy throughout the specimen. All 51 had MEPs that were located in the immediate vicinity of the IBF in all cases. 3 SMs also had ≤ 3 MEPs in one random normal parenchyma block. The mean numbers of MEPs and DCIS ducts/ SM were 13.15 and 8.98, respectively. 39% of MEPs were located within the area of the invasive CA or DCIS, 24% were in the 8 mm parenchymal rim beyond the IBF, 22% were 8 mm – 2 cm from the IBF outer edge, and 14% were 2.0 – 3.0 cm from the IIBF. 32 IBFs (62.7%) were clonal related to the initial CA. The mean number of MEPs in SMs was similar in clonal IBFs and second-primary IBFs. The range of MEPs cytologic features was narrower and more often cytologically similar to the IBF in clonal IBFs.

Conclusions: If persistent CA is source of IBFs, then SM's should be devoid of MEPs. Also, If MEPs were RT resistant, then they would have been present throughout the SM. However, we found neither: MEPs were present in all SMs. MEPs were localized to the area of the IBF and the remaining parenchyma had marked RT-lobular atrophy. This supports our ideas that: 1) MEPs are the pool of partially transformed precursor lesions that give rise to CA. 2) Most IBFs arise from MEPs that reemerge after RT. 3) RT works to reduce IBF risk, including in patients with negative margins by primarily eradicating MEPs.

138 Impact of Chromosome 17/HER-2 Polysomy on Reliability of Chromogenic In Situ Hybridization (CISH): A Study of Two Institutions

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Background: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the two assays currently used to determine HER-2 status of breast cancer, with FISH considered the gold standard. Chromogenic in situ hybridization (CISH) recently emerged as a potential alternative to FISH. We herin evaluated the performance of CISH on tumors showing chromosome 17 polysomy.

Design: HER-2 status was examined on duplicate sections of 286 breast carcinomas at sites A (MD Anderson) and B (Tampere) using FISH (Vysis kit). Polysomy of chromosome 17 was defined with FISH as the presence of three or more CEP17 signals in >10% of the tumor cells evaluated (required at site A) or in >30% of the tumor cells evaluated (required at site B). The performance of CISH (Invitrogen kit) on tumors with polysomy was evaluated by comparing CISH results with corresponding FISH results at each site. Results were interpreted by pathologists at each site according to manufacturers' guidelines.

Results: FISH results were available on 273 of the 286 (95.5%) tumors at site A, and 51 (18.7%) of them showed chromosome 17 polysomy. Among those 51 polysomy tumors, HER-2 gene amplification, non-amplification, and invalid results were found in 14 (27.5%), 37 (72.5%), and 0 tumors with FISH and in 12 (23.5%), 36 (70.6%) and 3 (5.9%) tumors with CISH, respectively. Agreement between CISH and FISH at site A was 0.96, and Cohen's kappa coefficient was 0.89. At site B, FISH results were available on 278 (97.2%) tumors, and 22 (7.9%) of them showed chromosome 17 polysomy. Among those 22 tumors, HER-2 gene amplification and non-amplification results were found in 12 (54.5%) and 10 (45.5%) tumors with FISH and in 13 (59.1%) and 9 (40.9%) tumors with CISH, respectively. There was no invalid result with FISH or CISH in site B. Agreement between CISH and FISH at site B was 0.96, and Cohen's kappa coefficient was 0.91.

Conclusions: The detection rate of chromosome 17 polysomy on the same tumor set varied substantially between the two test sites, possibly because percentage of tumor cells required for polysomy at each site was different. At each site, the incidence of HER-2 gene amplification in polysomy cases with CISH was comparable to that with FISH and the agreement of the two methods was excellent.

139 Effect of Immunohistochemistry (IHC)-Equivocal Breast Carcinomas on Performance of Chromogenic In Situ Hybridization (CISH) and Inter-Site Reproducibility

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Background: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the two assays currently used to determine HER-2 status of breast cancer, with FISH considered the gold standard. Chromogenic in situ hybridization (CISH) recently emerged as a potential alternative to FISH. However, the effect of IHC-equivocal cases on CISH results has not been well studied.

Design: We studied three sets of breast carcinomas. Set 1 contained 60 tumors with equivocal/2+ IHC score (antibody AB8, Neomarkers) during patient care at MD Anderson (site A). Set 2 contained 21 equivocal tumors determined by Herceptest (Dako kit) plus all 60 tumors from set 1. Set 3 contained all the Herceptest-equivocal tumors from Set 2. FISH (Vysis kit) and CISH (Invitrogen kit) assays were performed on duplicate tumor sections at sites A and B (Tampere). Results were interpreted by pathologists at each site according to manufacturers' guidelines.

Results: Of the 60 IHC-equivocal tumors in set 1, Herceptest scores of 0, 1+, 2+, 3+, and NA were found in 43%, 12%, 25% (n=15),13%, and 7% at site A and in 23%, 15%, 37% (n=22), 20%, and 5% at site B, respectively. Inter-site agreement on Herceptest results for set 1 was 0.86, and Cohen's kappa coefficient was 0.52. Gene amplification by FISH was found in 13% of the 60 tumors at site A and 15% at site B, and by CISH, in 10% at site A and 15% at site B. Agreement between FISH and CISH in the three datasets at each site is shown below:

	Set 1		Set 2		Set 3	
Test site	A	B	A	B	A	B
Tumors (n)*	54	56	73	76	34	41
Agreement	0.96	0.93	0.95	0.92	0.88	0.95
Cohen's kappa coefficientκ	0.84	0.74	0.79	0.72	0.54	0.77

* Tumors that have invalid FISH or CISH results were excluded.

Conclusions: Reproducibility of IHC-equivocal results was low when different antibody was used. Inter-site agreement on HER-2 status was only fair using Herceptest to reproduce equivocal score determined with different antibody. Agreement between CISH and FISH on IHC-equivocal cases was good to strong at each test site, and a lower degree of agreement tended to be seen in datasets containing a higher proportion of equivocal tumors determined by Herceptest.

140 Chromogenic In Situ Hybridization (CISH) Is a Reliable Method for Detecting HER-2 Gene Status in Breast Cancer: A Multicenter Study Comparing Three Methods

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Background: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the two assays currently used to determine HER-2 status in routine practice, with FISH considered the gold standard. Chromogenic in situ hybridization (CISH) recently emerged as a potential alternative to FISH. In this study, we evaluated the accuracy of CISH and the correlation between CISH, FISH and IHC.

Design: A total of 226 breast cancer specimens were consecutively obtained from two test sites. HER-2 status was determined using IHC (Herceptest; Dako kit), FISH (Vysis kit), and CISH (Invitrogen kit). IHC was performed at each site on tumors obtained from that site: 110 at site A (MD Anderson) and 116 at site B (Tampere). To determine inter-site reproducibility, FISH was performed at sites A and B on duplicate sections (n=226), and CISH at sites A, B, and C (Invitrogen) on triplicate sections (n=226). Results were interpreted by pathologists at each site according to manufacturers' guidelines.

Results: Of 221 tumors with available IHC results, 0, 1+, 2+, and 3+ scores were observed in 63.8%, 8.6%, 9.5%, and 18.1% tumors, respectively. The corresponding FISH results demonstrated gene amplification in 0.7%, 0%, 23.8%, and 81.6% tumors, respectively, and, by CISH, in 0.8%, 0%, 15.8%, and 84.2% tumors, respectively. Among three methods, the agreement and Cohen's kappa coefficients on HER-2 status were 0.95 and 0.84 between IHC and CISH, 0.94 and 0.79 between IHC and FISH, and 0.99 and 0.97 between CISH and FISH. Similar concordances were found when stratifying by tumor source. Finally, the inter-site reproducibility of CISH results on 221 tumors was excellent; the agreement and Cohen's kappa coefficients were 0.99 and 0.97 between sites A and B, 0.99 and 0.95 between sites B and C, and 0.98 and 0.93 between sites A and C. The agreement of FISH results between sites A and B was 0.99, and the kappa coefficient was 0.97.

Conclusions: High agreement in HER-2 status was found between the three methods, and near-perfect agreement was noted between CISH and the corresponding FISH results. Excellent inter-site reproducibility was found with FISH and CISH results.

141 Choice of Primary Anti-HER2 Antibody Affects Concordance of Immunohistochemistry (IHC) with Fluorescence In Situ Hybridization (FISH) for Determination of Breast Cancer HER2 Status

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Background: Accurate HER2 testing is critical to the selection of appropriate patients for treatment with trastuzumab as well as other HER2 targeted therapies. Recently published ASCO-CAP Guidelines require laboratories to document, in their validation of HER2 IHC, a high level of concordance (95%) between cases interpreted as positive or negative by both IHC and FISH. We wished to test the hypothesis that IHC and FISH

concordance could be related to the choice of primary anti-HER2 antibody, comparing a standard rabbit polyclonal anti-HER2 antibody with a new rabbit monoclonal anti-HER2 antibody.
Design: A series of 416 breast carcinomas had parallel HER2 analysis performed by IHC, using both the A0485 rabbit polyclonal antibody (Dako), and the SP3 rabbit monoclonal antibody (LabVision), as well as HER2 analysis by FISH using the PathVysion kit (Abbott Vysis). An IHC score of 3+ was considered positive, 2+ equivocal, and scores of 0 or 1+ were considered negative; FISH ratios of >2.2 were considered positive for amplification.
Results: Overall concordance between IHC negative (0, 1+) and FISH nonamplified cases, and between IHC positive (3+) and FISH amplified cases, were identical with the A0485 and the SP3 (>95% for positives, >98% for negatives). However, in 88 nonamplified cases, SP3 yielded 1+ results vs. equivocal (2+) with A0485, and in 17 amplified cases SP3 yielded 3+ results compared with equivocal (2+) with A0485. Three amplified cases yielded 2+ results with A0485 but 1+ results with SP3, and one amplified case yielded 1+ results with A0485 but 2+ results with SP3.
Conclusions: In our study of 416 breast cancer cases, the choice of anti-HER2 antibody did not affect the overall concordance between HER2 status as determined by IHC and FISH. However, significant numbers of nonamplified cases that yielded equivocal (2+) results with A0485 yielded negative (1+) results with SP3, and significant numbers of amplified cases yielded equivocal (2+) results with A0485 and positive (3+) results with SP3. This suggests that the use of SP3 might significantly reduce the number of IHC cases that would be submitted for FISH testing, while at the same time resulting in a very small number of cases yielding false negative IHC results.

142 Identification of Novel Clinical Phenotypes of Breast Cancer by Immunohistochemical Analysis

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Background: Breast cancer is a heterogeneous disease, of which several forms have been identified on the basis of their gene expression characteristics. We have previously demonstrated that protein expression characteristics can be used to identify comparable classes. In this study we extend this approach using improved computational and biostatistical methods to confirm the validity of such an approach and further define the key criteria for class membership.
Design: Expression of twenty-five proteins, with known relevance to breast cancer, have been assessed in a series of 1,076 patients. This large data set has been examined by four alternative computational data clustering techniques. Concordance between techniques was used to elucidate core classes of patients which could be well characterised.
Results: 663 (62%) of the 1076 patients were assigned to six different core classes, while 413 (38%) patients were of indeterminate or mixed class. Three of these core classes correspond to well known clinical phenotypes (luminal A, luminal B and HER2). Two classes correspond to the well known basal phenotype, but exhibit a novel differentiation into two sub-groups. The last class appears to characterise a novel luminal sub-group.
Conclusions: This study serves to confirm that key clinical phenotypes of breast cancer can be identified. It has identified that both the luminal and basal breast cancer phenotypes appear to be heterogeneous and contain distinct sub-groups. The six clinical phenotypes determined in this study are a new luminal group, luminal-N, the new basal sub-groups, basal p53 altered and basal p53 normal, and the previously well-established luminal A, luminal B and HER2 groups.

143 Successful Design of Low-Level Estrogen Receptor Positive Tissue Controls for Critical Quality Control Measures

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Background: While it is easy to identify human tissues with high levels of estrogen receptor (ER) expression by immunohistochemistry (IHC), it is important to incorporate positive controls with low ER levels to adequately detect subtle drop of the sensitivity of ER IHC assay. However, most benign gynecologic tissues and ER-positive breast cancers express high levels of ER, which are not suitable as low-level positive tissue controls. This limits the tools to measure early and subtle reduction of the IHC assay. Therefore, it would be convenient to utilize these commonly encountered gynecologic specimens and artificially induce ER signal reduction by intentionally manipulating the pre-analytical parameters of the assay.
Design: 5 Hysterectomy specimens were harvested and fixed in 10% neurtal buffered formaldehyde at varying intervals to determine whether the level of hormone expression could be artificially lowered. Samples of endomyometrium, cervix, leiomyoma, and ovary were harvested from these specimens. For each tissue, four samples were prepared and fixed at intervals of 12 to 24 hours, one week, one month, and 3 to 5 months (Total blocks = 62). Paraffin-embedded tissues were evaluated for ER expression (ER rabbit monoclonal antibody, SPI, Lab Vision, Fremont, CA, USA). Tissue pretreatment was achieved by heating the sections in citrate buffer (pH = 6) in a scientific pressure cooker. Following primary antibody incubation (30 minutes) in automation IHC stainer (362D, Lab Vision), A two-step polymer detection system (Advance; Dako, Carpeteria, CA, USA) was used, followed by 5-minute DAB incubation.
Results: No significant signal reduction was observed in samples subjected to fixation times of ≤ one month. On the other hand, formalin fixation of three to five months produced a measurable loss of ER expression in all the examined tissues, ranging from 20% to 60% reduction of both the fraction of positive cells as well as signal intensity, with the most dramatic decrease identified in the ovarian samples.
Conclusions: This study provides a useful tool to artificially reduce ER levels in readily accessible tissues such as hysterectomy specimens, rendering this technique convenient for the community pathologist. Fixation of gynecologic tissues for greater than three months decreases estrogen receptor expression, making it useful as a low-level positive control for immunohistochemical assays, which is of utmost importance in monitoring this critical predictive assay.

144 Measuring Extent of DCIS in Breast Excision Specimens: A Comparison of Four Methods

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Background: Measuring the extent of non-palpable DCIS in a breast specimen is challenging. An accurate assessment of the size of DCIS is important as it influences patient management. Several methods for estimating the extent of DCIS have been used, but none have been validated. The aim of this study was to compare the extent of DCIS using various methods of estimation.
Design: 78 primary breast excisions with DCIS and an accompanying sliced specimen radiograph were retrospectively reviewed. All specimens had been sampled using the serial sequential method, which involved mapping the location of each block on the sliced specimen radiograph and calculating extent through 3-D reconstruction. This method was considered the gold standard. The other methods included: 1) calculating the extent based on sampling only areas of calcification (calcification method); 2) recording the number of blocks involved by DCIS and multiplying this by 0.3 cm (blocks method); 3) measuring the largest extent of DCIS on a single slide (single slide method). Data was analysed by characterizing the under- and overestimation relative to the serial sequential method, and by calculating the percentage correctly classified into size categories.
Results: There were 4 grade I (5.1%), 40 grade II (51.3%) and 34 grade III (43.6%) cases. By the serial sequential method, the mean number of blocks submitted was 26.7 (SD 11.0). All three alternative methods tended to underestimate the DCIS, with median underestimates of zero when extent (according to the serial sequential method) was <0.5cm, increasing to 1.2cm, 1.6cm, and 3.7cm for the calcification, blocks and single slide methods when extent was >4cm. Table 1 shows the number of cases in each clinically significant size category by the serial sequential method with comparison to the number (%) of cases classified into the same group by alternate methods.

Table 1

	Serial sequential	Calcification method	Blocks method	Single slide method
<0.5cm	4	4 (100)	4 (100)	4 (100)
0.5-2cm	34	28 (82.4)	28 (82.4)	28 (82.4)
>2-4cm	26	19 (73.1)	14 (53.8)	1 (3.8)
>4cm	14	9 (64.3)	8 (57.1)	0 (0)
Total	78	65 (83)	54 69)	33 (42)

Conclusions: When DCIS is <0.5cm, all methods of measuring extent are equivalent. For sizes >0.5cm the alternative methods tended to underestimate the DCIS extent relative to the serial sequential method with the underestimation becoming more pronounced as size increased. These size discrepancies are of clinical relevance.

145 Quantification of Regulatory T-Cells in Sentinel Lymph Nodes of Breast Cancer Patients

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Background: Availability of new markers to characterize the lymphocytic infiltrate has generated renewed interest in studying the host immune response to cancer. Regulatory T cells (Treg) are a subset of CD4 T-cells with potent immunosuppressive function that can be identified by the expression of the nuclear transcription factor, Forkhead box P3 (FOXP3). Recent studies have shown that there is a significant correlation between higher numbers of infiltrating Treg and worse outcome in breast and other cancers. Alterations in the percentages of T cells (CD4/CD8) have been reported in the axillary lymph nodes (ALN) of breast cancer patients, and it has been suggested that they reflect tumor-induced dysregulation of the regional immune system. However, no data are available about the numbers of Treg and their relationship to ALN metastases. Here we quantified Treg in sentinel lymph nodes (SLN) of breast cancer patients with different nodal status.
Design: Sentinel lymph nodes from 34 patients with invasive breast carcinoma were studied. The nodal status was (a) negative (pN0), N=10 (b) negative but with isolated tumor cells (ITC) < 0.2 mm (pN0(i+)), N=9 (c) metastases >0.2mm (pN1), N=15 (0.2-2mm (N=6), >0.2 (N=9). Immunohistochemistry (IHC) was performed in formalin-fixed paraffin embedded sections with antibody to FOXP3 (e-Biosciences) to identify Tregs in the lymph nodes. Normal tonsils were used as positive control. The number of lymphocytes with positive nuclear staining for FOXP3 in the interfollicular areas was counted independently by two pathologists in 5 high power field (HPF, 60X).
Results: Overall, the number of FOXP3+ cells was higher in the SLN with metastases (median=120, SD=27; range: 93-147) than in negative SLN (median=96, SD=53; range: 43-149). SLN containing micro and macro metastasis showed similar number of Tregs. The SLN with ITC had a median Treg number similar to SLN with metastases (median=119), however there was a high degree of variability (SD=68; Range: 51-187).
Conclusions: FOXP3 immunohistochemistry allows for easy and reproducible identification of Treg cells in SLN of breast cancer patients. Initial data suggest that the number of Treg may be lower in negative SLN as compared to SLN with metastases. This is consistent with the hypothesis that a tolerogenic/immunosuppressive environment may favor unrestricted tumor growth. SLN with ITC showed high degree of variability in the number of Treg. Extension of this study to a larger number of cases will help us further understand the biology of metastasis in the lymph nodes.

146 Can Axillary Dissection Be Avoided in Patients with Sentinel Node Micrometastasis? The Role of Pathologic Assessment of Breast Tumors in Predicting Non-Sentinel Node Metastasis

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Background: The role of axillary dissection in patients with micrometastasis (MM) in sentinel lymph node biopsy (SLNB) remains controversial. Assessment of key pathologic features of the primary breast cancer may be useful in predicting non-sentinel lymph node (NSLN) metastasis and thus spare some patients the need for completion axillary dissection.

Design: We searched our database from 1997 through 2007 for cases of breast cancer for which SLNB was performed. We analyzed all positive sentinel node cases in which completion axillary dissection was performed. MM was defined as metastatic foci measuring 0.2 mm to 2 mm according to the Amercian Joint Committee on Cancer, 6th edition. All sentinel nodes were subjected to three levels of hematoxylin and eosin (H&E) with intervening levels stained with cytokeratin AE1/AE3. NSLNs were analyzed for metastasis by a single H&E slide. The primary breast tumor was analyzed for grade, tumor size, and lymphovascular invasion (LVI). Comparison of pathologic features of the tumor and the presence or absence of NSLN metastasis were determined. Statistical analysis was performed by using the Fischer exact test.

Results: Of 1933 SLNBs, 431 were considered positive. Of these, 100 (23%) cases were micrometastatic. Completion axillary dissection was performed in 68 patients in the MM group (68%). NSLN metastasis was seen in 13% of cases in the MM group. Analysis of primary tumor varialbes showed that the presence of LVI was the only significant parameter in predicting NSLN metastasis in MM group (see table).

Sentinel lymph node with micrometastasis

Non-sentinel LN status	pT1	pT2	LVI	Well Differentiated	Moderately Differentiated	Poorly Differentiated
Positive(9)	5 (64%)	3 (36%)	7 (88%)	3 (37%)	2 (25%)	3 (37%)
Negative(59)	46 (78%)	13 (22%)	37 (36%)	15 (25%)	17 (29%)	27 (46%)
p value	0.3	0.3	0.01	0.6	0.6	0.6

LN: Lymph node; LVI: Lymphovascular invasion

Conclusions: Metastasis to NSLN occurs in 13% of MM cases. LVI can help predict NSLN metastasis and guide completion axillary dissection.

147 Does the Ki-67 Proliferation Index Complement the Oncotype DX™ Recurrence Score?

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Background: The Oncotype Dx™ breast cancer assay is a RT-PCR-based genomic test that analyzes the expressions of 21 genes to quantify the risk of recurrent distant disease and to predict clinical benefits from chemotherapy in LN-negative, ER-positive patients who will receive tamoxifen treatment. Routine IHC stains for ER, PR and HER2/neu overlap with the gene panel, however, it additionally analyzes proliferation and invasion markers. The aim of this study was to investigate the correlation between the proliferation marker Ki-67 and the Oncotype Dx™ recurrence score ™ (RS) and to evaluate a potential complementing value.

Design: All 32 BC (ductal, lobular, mucinous, tubular) analyzed with the Oncotype Dx™ assay over a 2-year period were evaluated. ER, PR and HER2/neu results were available. IHC stain for Ki-67/MIB-1 was performed on the same paraffin block used for Oncotype analysis. The Ki-67 proliferation index (PI), evaluated in 200 tumor cells, and the Nottingham (NH) index were compared to the RS. In accordance with previous studies, RS < 18, 18-30, and >31 were considered low, intermediate and high-risk, respectively.

Results: All BC were LN-negative, ER, PR-positive and HER2/neu negative. The mean RS was 18.8 (4-30); the mean Ki-67 was 23.7% (range 3.6-66%). Of the 32 BC, 13 (mean 12.8, Ki-67=16.4%; range 3.6-36.4%) had a low RS and 19 (mean 23, Ki-67 25%; range 6.0-66.15%) had an intermediate RS. No case had a high RS. Comparison of the NH index with the RS and the Ki-67 PI revealed concordance. The mean RS for 22 well-differentiated BC was 16.3 (Ki-67=15%) and for 8 moderately-differentiated tumors 23.1 (Ki-67= 33%). The 2 poorly-differentiated carcinomas showed a mean RS of 29.5 (Ki-67=48%). RS and Ki-67 PI also correlated with the tumor morphology; mucinous and tubular carcinomas had significantly lower RS and Ki-67 PI than IDC and ILC.

Conclusions: A substantial number of cases for which Oncotype Dx analysis is sought are cases where additional information is needed to determine possible benefits from chemotherapy. However, 19 of 32 cases with an intermediate RS failed to provide this information. Furthermore, it has been established that even among tumors with low RS, about 7% do develop recurrences. The finding of occasional high Ki-67 index in BC with low RS scores may correspond to the 6% BCs with low RS that did recur. Therefore, we propose evaluation of a combination of RS and Ki-67 index to potentially further segregate carcinomas with recurrence potential from those in the low and intermediate RS.

148 Chromosome 7 Aneusomy in Metaplastic Breast Carcinomas with Chondroid, Squamous and Spindle-Cell Differentiation

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Background: A vast majority of metaplastic carcinomas (MC) of the breast have a triple negative immunoprofile and a high frequency of EGFR overexpression. The EGFR gene, located at 7p12, is a potential target for EGFR inhibitors, but the underlying genetic mechanism of the EGFR overexpression is unclear. Recent studies have found EGFR amplification by CISH in only 1/3 of MC of the breast. CGH array studies have noted a gene copy number gain of 7p11.2-tel. Activating mutations of the EGFR tyrosine kinase domain have not been identified. The aim of this study was to analyze the EGFR/CEP7gene copy number in different subtypes of MC of the breast.

Design: Seventeen MC with chondroid (n=10), squamous (n=4), and spindle-cell differentiation (n=3) and 2+/3+ EGFR overexpression by IHC were selected. FISH was performed on paraffin sections using the LSI® EGFR/CEP® 7 Dual Color Probe (Vysis). Signals were counted in 20-80 tumor cell nuclei, and the mean copy number for EGFR and chromosome 7 was determined. In accordance with previous studies, EGFR/CEP7 ratios 0.8-1.2, <0.8, and >1.2 were considered balanced, copy loss and copy gain, respectively. Amplification was defined as more than 5 signals in >50% of tumor cells or large gene copy clusters.

Results: The mean EGFR copy number was 2.3 (range 1.6-3.2); the mean CEP7 copy number was 2.4 (range 1.6-3.2). All cases had a balanced EGFR/CEP7 ratio (mean 1.0; range 0.9-1.1). EGFR amplification was not identified in any case. MC with chondroid differentiation revealed monosomy in 25% of cases (1/3 of tumor cells) and polysomy in 25% of cases (3-4 copies of EGFR/CEP7 in 1/2 of tumor cells). Trisomy was present in 50% of carcinomas with squamous differentiation and 100% of MC with spindle cell differentiation (1/2 of the tumor cells).

Conclusions: EGFR amplification is not the underlying mechanism in our cases; however, an EGFR gene copy number heterogeneity was noted in half of the MC. Comparison with the CEP7 copy number revealed monosomy / polysomy of chromosome 7 instead of losses / gains specific to the EGFR gene or 7p. In the absence of EGFR amplification, and the known rarity of EGFR activating mutations in breast cancer, chromosome 7 aneusomy might be a useful criteria for determining potential candidates for a clinical trial with EGFR tyrosine kinase inhibitors in breast carcinomas with chondroid, squamous and spindle cell differentiation.

149 Collagen α1 (XI) in Normal and Malignant Breast Tissue

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Background: Little is known about collagen XI expression in normal and malignant breast tissue. Tissue microarrays, constructed from 72 patients with breast carcinoma and matched normal tissue, were immunohistochemically stained with five antisera against isoform-specific regions of collagen α1(XI) amino terminal domain.

Design: Staining intensity was graded on a 0 to 3 scale in epithelial cytoplasm, stroma, and endothelial staining of the vasculature of each tissue core. The staining was compared to known pathologic parameters: age, tumor size, overall tumor grade, nuclear grade, histologic grade, mitotic rate, angiolymphatic invasion, node status, estrogen receptor status, progesterone receptor status, and HER-2/neu status. Estrogen and progesterone receptor status were used as a control for comparison.

Results: With antisera V1a and Npp, stroma surrounding cancerous cells was found to have decreased collagen α1(XI) staining compared to stroma adjacent to normal epithelium (p=0.0006, p=<0.0001). Collagen α1(XI) staining with V1a antiserum in cytoplasm of cancer cells demonstrated decreased intensity in metastasized primary tumors when compared to non-metastasized primary tumors (p=0.009). Intensity of cytoplasmic staining in cancerous tissue with V1a antiserum had an inverse relationship to tumor size (p=0.023). Cytoplasmic staining with V1a and Npp antisera in cancer demonstrated an inverse relationship to positive estrogen receptor status in cancer (p=0.022, p=0.012). Cytoplasmic staining for Npp in cancerous tissue exhibited an inverse relationship to progesterone receptor status (p=0.044). Stromal staining for Npp in cancerous tissue demonstrated an inverse relationship with histologic grade (p=0.015). Vascular endothelial staining for Npp in cancerous tissue was directly related to angiolymphatic invasion (p=0.006).

Conclusions: This is the first study to localize collagen XI within normal and malignant breast tissue. Collagen (α1)XI appears to be down regulated in stroma surrounding breast cancer. Detection of collagen XI in breast tissue may help predict women who have lymph node metastases.

150 Interobserver Variability in Histological Grading of Invasive Mammary Carcinoma as a Function of Subspecialization

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Background: Histological grade (HG) is a well-established prognostic factor for invasive mammary carcinoma (IMC). While many studies have examined the reproducibility of such grading, few have evaluated interobserver variability between specialty and general pathologists. Accordingly, the objective of this study was to determine the degree of interobserver variation in the assessment of HG for IMC among different groups of pathologists with varying levels of experience and subspecialty expertise.

Design: A representative section from 92 cases of IMC was selected to be reviewed by 10 observers at a single academic medical center, including 3 surgical pathology fellows, 3 breast pathologists (3-10 years of experience), and 4 non-breast pathologists (8-25 years of experience). The reviewers independently assigned a histologic grade to each case using their own interpretation of the modified Bloom-Richardson grading system without further instruction. Pair-wise kappa values were determined and then compared among the 3 groups of pathologists using the student t-test.

Results: Results are summarized in the Table. Overall, only 20% of the cases had a unanimous grade, with the breast pathologist group having the highest proportion of unanimous grades (61%), followed by the non-breast pathologist group (51%), and fellows (41%). As a group, interobserver agreement among the fellows was significantly lower than the other two groups (P < 0.05). The interobserver agreement among the

non-breast pathologists was also lower than that among the breast pathologists but the difference in mean kappa values only approached statistical significance (P = 0.07).

	Overall	Fellows	Breast Pathologists	Non-Breast Pathologists
No of cases with unaninous agreement	18	38	56	47
No of cases with 3-way disagreement*	10	3	0	0
Mean Kappa value (SD)	0.47 (0.13)	0.34 (0.02)	0.57 (0.14)	0.49 (0.08)

* all 3 grades were reported for the same individual case.

Conclusions: Overall, interobserver agreement in histological grading of IMC was fair. As a group, breast pathologists showed the least variability in evaluating HG. This suggests that more consistent results can be achieved if such evaluation is limited to breast pathologists, both for routine patient care and also for emerging studies comparing HG to potentially new predictor or prognostic markers for breast cancer.

151 Using a Higher Cutoff for the Percentage of HER2-Positive Cells Decreases Interobserver Variability in the Interpretation of HER2 Immunohistochemistry (IHC)

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Background: A new interpretation guideline for HER2 IHC in breast carcinoma (BC) has been the requirement for uniform circumferential staining in at least 30% (in contrast to 10%) of tumor cells for a result to be considered positive. A previous study has shown that using such a cutoff improves concordance with FISH; however, the effect of this, if any, on the interobserver variability in the interpretation of HER2 IHC has not been evaluated.

Design: HER2 immunostained sections from 96 cases of BC were reviewed by 10 pathologists at a single academic medical center. Cases were scored as positive (3+) when moderate to strong circumferential membranous staining was identified in at least 10% of tumor cells; the actual percentage of cells with such staining was also estimated. Agreement rates between different pathologists and pairwise Kappa values (n = 45 each) at the 10% cutoff were compared to those at the 30% cutoff using the paired samples T-test.

Results: The agreement rates between pathologists (Table 1) and the Kappa values (Table 2) using the 30% cutoff (upper right in both tables) were higher than those using the 10% cutoff (lower left in both tables) in 28 and 30 of the 45 comparisons, repectively (these are shaded in both tables). The average interobserver agreement rates and kappa values were 70% (range, 47-82%) and 0.49 (range, 0.18-0.7) using the 10% cutoff, and 72% (range, 54-85%) and 0.54 (range, 0.24-0.77), respectively, at the 30% cutoff (both P values = 0.001).

Conclusions: Using a 30% cutoff for the percentage of HER2-positive cells by IHC decreases interobserver variability in the interpretation of HER2 IHC in BC; additional analysis stratified by experience and expertise is ongoing.

Table 1: Agreement rates

Pathologist	A	B	C	D	E	F	G	H	I	J
A		82%	69%	79%	69%	75%	80%	78%	78%	74%
B	79%		64%	74%	72%	78%	81%	84%	79%	71%
C	68%	62%		73%	73%	54%	62%	59%	62%	78%
D	79%	75%	66%		73%	65%	70%	68%	72%	72%
E	65%	61%	78%	69%		60%	62%	66%	68%	80%
F	69%	73%	47%	65%	48%		72%	82%	78%	60%
G	71%	73%	66%	69%	69%	69%		81%	83%	65%
H	72%	80%	52%	68%	68%	82%	72%		83%	63%
I	71%	77%	57%	71%	71%	77%	73%	82%		67%
J	78%	76%	77%	72%	72%	60%	70%	63%	68%	

Table 2: Pairwise Kappa values

Pathologist	A	B	C	D	E	F	G	H	I	J
A		0.73	0.53	0.70	0.50	0.50	0.67	0.61	0.63	0.60
B	0.68		0.43	0.58	0.56	0.54	0.68	0.77	0.63	0.54
C	0.51	0.42		0.59	0.59	0.24	0.40	0.33	0.40	0.69
D	0.70	0.61	0.45		0.58	0.37	0.50	0.45	0.54	0.56
E	0.45	0.39	0.66	0.51		0.28	0.35	0.43	0.47	0.73
F	0.41	0.45	0.18	0.37	0.19		0.65	0.62	0.54	0.31
G	0.52	0.55	0.47	0.49	0.52	0.43		0.65	0.73	0.42
H	0.50	0.66	0.25	0.45	0.26	0.62	0.51		0.72	0.38
I	0.49	0.61	0.32	0.52	0.37	0.52	0.55	0.69		0.46
J	0.69	0.65	0.67	0.56	0.60	0.31	0.52	0.38	0.48	

152 A Multinstitutional Study Comparing the Oncotype-DX Recurrence Rates with Standard Clinical and Pathological Features of Breast Cancer, the Nottingham Prognostic Index (NPI) and ADJUVANT! Online

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Background: Although its use is on the rise, it is not entirely clear how well the recurrence scores (RS) and predicted 10-yr recurrence rates (RR) obtained with the Oncotype-DX (ONC) assay compare with standard pathological features of breast cancer (BC), and especially with prognostic scores based on these features. One such score, the Nottingham Prognostic Index (NPI), has been widely tested and used in Europe and also stratifies patients into different risk groups (NGs), while ADJUVANT! (ADJ) is a web-based program that uses pathological features and models derived from the published literature to predict 10-yr RRs of BC. The aim of this study was to compare elements of the ONC assay with standard pathological features of BC, as well as with the NPI score, NGs and ADJ RRs.

Design: The pathological features of cases of BC with available ONC results were used to calculate the NPI [(0.2 x tumor size + histological grade (HG)], based upon which the cases were divided into 3 NGs: one with an NPI ranging from 2.08 to 2.4, another ranging from 2.42 to 3.4, and a third ranging from 3.42 to 4.4, as previously reported. The clinical and pathological findings were also used to calculate the predicted ADJ RRs online. The relationship between the different parameters was assessed using the Pearson correlation coefficient (r).

Results: Three hundred and seventy four cases (from 8 institutions) have been collected to date. Grade 3 tumors were more frequently seen in patients with high risk ONC RSs (46% of cases vs. 12% and 17% of cases with intermediate and low RSs, respectively; P < 0.001). There was a significant correlation between the predicted ONC RR and the HG (r = 0.236; P < 0.001), the NPI (r = 231; P < 0.001), the NG (r = 0.168; P = 0.015), and the predicted ADJ RR (r = 0.146; P = 0.015), but not with age or tumor size.

Conclusions: There is a statistically significant correlation between elements of the ONC assay and HG as well as with prognostic scores that are partly based on grade. Accrual of additional cases and further statistical analysis is being performed.

153 A Comparative Histopathological and Clinical Study of "Triple-Negative" Invasive Mammary Carcinomas in African American and Caucasian Women

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Background: Breast cancer is the most common malignancy among African American (AA) women. AA women are more likely to be diagnosed with late stage, highly aggressive, rapidly growing and less hormone responsive tumors, and are more likely to die from the disease. Underlying causes of racial differences in breast cancer are not all clearly known. An aggressive rare subtype of cancer known as Triple negative (TN), also refered to as basal-like [negative for estrogen receptor (ER), progesterone receptor (PR) and Her2] is reported to be more common in AA, <40 yrs old. These tumors are associated with late stage at diagnosis and shorter survival. We examined clinical and histopathologic features and prognostic markers of TN tumors in AA and Caucasian women.

Design: TN tumors from 143 patients (24 AA and 119 Caucasian) were studied. Characteristics included tumor size, grade, histologic type, angiolymphatic invasion, lymph node (LN), ER, PR, p53, EGFR, BCL2, Her2, p27, p21 and ploidy status were analyzed. TN status was defined as ER and PR < 10% and Her2 negative by FISH and immunohistochemistry. Overall survival for 13 year duration was also evaluated.

Results: 24/143 (16.8%) TN tumors were found to occur in AA women. 79% of the TN in AA were invasive ductal compared to 88% in Caucasians. Most of the TN tumors were SBR grade II (29% AA vs. 19% Caucasian) and grade III (67% and 75%). Compared to Caucasian-TN tumors, AA TN tumors were more likely to be diagnosed in women under 50 years, larger, with higher rate of lymphovascular invasion, and more likely to be LN+. The frequency of EGFR, p53 and Bcl-2 positive TN tumors was slightly higher in AA than Caucasians (76% vs. 64%, 63% vs. 51% and 25% vs. 23%, respectively), lower for p27 and p21, with no difference in ploidy status between AA and Caucasians. Higher MIB-1 scores were noted in both groups (median of 51% for AA vs. 44% for Caucasians). The Overall survival was almost identical in both groups (alive 83% in AA, 85% in Caucasians).

Conclusions: TN tumors represent a unique subset of breast cancer that have a characteristic genetic profile and are associated with poor outcome. While TN tumors from AA and white women in our study had minor histopathologic and biomarker differences, we saw no difference in overall survival. Further, characterization of these tumors may assist in unraveling racial differences in breast cancer and in developing of better targeted treatment strategies.

154 Significance of Sentinel Lymph Node Positivity in Patients with a Core Biopsy Diagnosis of DCIS

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Background: Ductal carcinoma in-situ (DCIS) is defined as a clonal proliferation of malignant epithelial cells confined to the ductal-lobular units without invasion through the basement membrane. Theoretically the incidence of metastasis to regional lymph nodes should be zero. The role of sentinel lymph node biopsy (SLNB) in patients with a core biopsy diagnosis of DCIS is still controversial. An argument in favor of SLNB is that a significant number of these patients will have invasive carcinoma (IC) on excision.

Design: 141 patients with a core biopsy diagnosis of DCIS between January 2004 to July 2007 were retrieved from the Laboratory Information System of the Department of Pathology, UT Southwestern Medical Center, Dallas, Texas.The aim of this study was to determine: 1) the incidence of IC in patients with biopsy diagnosis of DCIS; 2) incidence of SLN metastasis in this group and also in patients with a final diagnosis of DCIS; 3) the significance of SLN positivity in DCIS and its impact on clinical management.

Results: 107/141 had follow-up excision. Invasive carcinoma (IC) was present in 25/107 (23%) microinvasion in 7 (6.5%), DCIS in 64 (60%), ADH in 4 (3.7%), no residual tumor in 7 (6.5%). 184 SLNs from 64 patients were sampled at the time of definitive surgery. Nine of the 64 cases (14.0%) showed SLN metastasis, (5 macromet, 2 micromet and 4 isolated tumor cells). Six of the 9 (67%) patients with positive SLN had IC and 3 (33%) showed pure DCIS on excision. Five of the 6 ICs showed extensive DCIS. Three of 37 (8.1%) patients with a final diagnosis of DCIS had positive SLN (2 macromet, 1 micromet). Extensive sampling did not reveal IC. ALND was performed in 6/9 cases (67%) and no additional positive lymph node noted.

Conclusions: The prevalence of SLN metastasis in patients with a biopsy diagnosis of DCIS was 14% and the majority had IC on excision, 83% had extensive DCIS associated with the IC. Positive SLN was noted in 8.1% with a final diagnosis of DCIS. We believe that patients with DCIS with positive SLN should be presumed to have occult invasive disease and staged as node positive invasive cancers.

155 Are ER and HER2/neu the Only Independent Markers of Overall Survival?

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Background: Management of breast cancer to date is based on risk categories (low, medium, high) as outlined in the St. Gallen guidelines 2007. The categories are based on a set of pathological and clinical parameters and include ER, PR and Her2. Cluster analysis has produced data to classify breast cancer into five classes (luminal A, B normal like, Her2/neu positive and basal types). There is substantial variability in disease outcome within each risk category. These limitations require the assessment of relevant prognostic and predictive biological markers using a large cohort of breast cancer patients with long term follow up.

Design: Tissue microarrays were constructed using 1mm core in triplicates from 958 cases from the Henrietta Banting database of well characterized cases with a mean follow up of 8.5 years, treated in a single institution. Commercially available antibodies to ER, PR, Her2/neu, EGFR, p53, Ki67, CK5/6, cyclin D1 and TopoII were used for immunohistochemistry. The markers were assessed as categorical variables. Univariate and multivariate analysis examined the association between the markers and overall survival.

Results: Within the follow up period 39.6% of the patients died. The mean time to death was 5.3 years. When each of the markers was examined individually, after adjustment for age, tumour size, grade and nodal status, we found significant associations between survival and Her2/neu (RR = 1.31), EGFR (RR = 1.38), TopoII (RR =1.29) Ki67 (RR = 1.28) and CK5/6 (RR = 1.42). Cyclin D1 and p53 were not significant. However, when all the markers were entered to the survival model together with size, grade age and nodal status, only ER (RR 0.74 CI 0.6-0.92; p = 0.006) and Her2/neu (RR 1.26 CI 1.01-1.57 p=0.04) emerged as independent prognostic factors for overall survival. Basal phenotype (triple negative and CK5/6 or EGFR positive) was associated with significantly increased mortality compared to non basal phenotype (p=0.0007)

Conclusions: This study confirms the prognostic value of ER and Her2/neu in predicting survival in a large cohort, confirming the criteria used for the St Gallen guidelines. Strong interrelations between the markers and their association with tumor grade limit their significance as independent prognostic markers in this cohort. Assessment of EGFR and CK5/6 in triple negative tumors should be considered in the routine management of breast cancer, not only to aggressively manage this subset of patients but to investigate new treatment modalities.

156 MyD88 Expression in Breast Cancer Is Associated with Poor Overall Survival Using an Objective and Quantitative (AQUA) Method of Analysis

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Background: MyD88 an adaptor molecule in the interlukin-1 (IL-1) signaling pathway has been recently shown to play a critical role in colorectal, liver and ovarian tumorogenesis by production of cytokines (IL-6,IL-12 and Tumor necrosis factor alpha) and chemokines through the Toll-like receptor (TLR) pathway. We investigated the prognostic significance of MyD88 protein expression in node positive and node negative breast cancers in tissue microarrays (TMAs) using AQUA.

Design: MyD88 expression was studied by fluorescent immunohistochemical staining of a breast TMA (670 breast cancers) using anti MyD88 antibody and analyzed by an objective method (AQUA). MyD88 protein expression was measured at two fold redundancy with high reproducibility. Cytokeratin was used to identify tumor cells. Fluorescent chromogen Cy-5-tyramide was used for MyD88 identification. Optimal cut-points for the training set were selected using the X-tile software and statistical analysis was done using Statview.

Results: MyD88 showed non-nuclear (cytoplasmic) localization with raw AQUA scores ranging from 3.55 - 173.4 (mean 47.4). Regression between slides from the same block and from different TMA blocks (from the same patient) showed high correlation for MyD88 expression (R= 0.94, and 0.68) respectively. The average AQUA score for MyD88 expression in 582 analyzable tumors was used in Cox univariate survival analysis, where high MyD88 expression was associated with poor patient outcome (log rank p = 0.0002). Binarizing the data using a training set-derived cut-point of 79.7 was applied on the validation set (n=272) showing that high MyD88 expression was associated with poor overall survival (validation set log rank $p < 0.005$) by Kaplan-Meier analysis. When subclassified by nodal status, high MyD88 was associated with poor overall survival in the node positive (n= 136) (p= 0.005) and ER negative patient subsets (n=138)(p= 0.02) but not in the node negative (p= 0.94) and ER positive patient subsets (p= 0.96). MyD88 expression retained its independent association with survival by multivariate analyses (p=0.014).

Conclusions: High MyD88 expression in breast cancer is associated with worse overall survival. Its expression in breast cancers could be especially valuable in node positive and ER negative patient subsets. Additionally, MyD88 expression in breast cancers may represent a potential new target in predicting response to biomodulaters.

157 Clinical Significance of Pleomorphic Lobular Carcinoma In Situ (PLCIS) on Core Needle Biopsies with Surgical Excisional Follow-Up

DM Haynik, M Chivukula, A Brufsky, DJ Dabbs, G Carter. Magee Women's Hopsital, Pittsburgh, PA; Magee Women's Hospital, Pittsburgh, PA.

Background: Lobular carcinoma in situ (LCIS) of the breast is generally considered an indicator for an increased risk of invasive breast cancer with equal predisposition in either breast. Pleomorphic LCIS (PLCIS) is a recently characterized entity that exhibits both ductal and lobular features associated with more aggressive features compared to classical LCIS. Current recommendations are to treat this entity as high grade ductal carcinoma in situ (DCIS). The evolution of PLCIS is poorly understood, and clinical data on the behavior of this entity is lacking. There is also a dearth of data regarding associated lesions and putative precursors seen with PLCIS.

Design: We reviewed 10 cases of PLCIS diagnosed on core needle biopsy (CNB), along with the subsequent surgical resection (SR), from the surgical pathology files of Magee Women's Hospital. The cases were diagnosed from 2002-2007. The slides from the CNB as well as SR were reviewed independently by three pathologists.

Results: Age range was 45-77 with a mean of 61.4 yrs. Nine patients underwent biopsy for calcifications and one patient for a mass lesion. All patients subsequently underwent excisional procedure (1 excision biopsy, 1 lumpectomy, 6 segmental mastectomies, and 2 simple mastectomies). Histologically, PLCIS showed dyscohesive cells with grade 3 nuclei, prominent nucleoli, and moderate to abundant eosinophilic or vacuolated cytoplasm. Nine cases had central comedo necrosis and nine had associated microcalcifications. Residual PLCIS was found on excisional procedures in 100% (10/10) of cases. Invasive lobular carcinoma was found in 30% (3/10) of cases. Other lesions associated with PLCIS were classical atypical lobular hyperplasia -90% (9/10), ductal carcinoma in situ-20% (2/10, nuclear grade 2 and nuclear grade 3), atypical ductal hyperplasia- 40% (4/10), fibrocystic changes-90% (9/10), sclerosing adenosis- 40% (4/10), columnar cell change-30% (3/10), radial scar-10% (1/10), papilloma-20% (2/10) and pseudoangiomatous stromal hyperplasia-10% (1/10).

Conclusions: 1. The incidence of upstaging PLCIS on CNB to invasive carcinoma is thirty percent. 2. Atypical lobular hyperplasia of classic type is a particularly common companion of PLCIS, suggesting that the origin of PLCIS may be the same neoplastic stem cells that evolve into classical lobular neoplasia.

158 Biophenotype of Pleomorphic Lobular Carcinoma In Situ on Core Needle Biopsies

DM Haynik, M Chivukula, A Brufsky, G Carter, DJ Dabbs. Magee Woman's Hospital, Pittsburgh, PA; Magee Woman's Hopsital, Pittsburgh, PA.

Background: Lobular carcinoma in situ (LCIS) of the breast is generally considered an indicator for an increased risk of invasive breast cancer with equal predisposition in either breast. Pleomorphic LCIS (PLCIS) is a recently characterized entity that exhibits a hybrid of both ductal and lobular features. Current recommendations are to treat this entity as a high grade ductal carcinoma in situ (DCIS), however, clinical data on the behavior of this entity is lacking. The aim of our study is to evaluate the histologic and immunohistologic profile of PLCIS and present clinical follow-up data.

Design: We reviewed 10 cases of PLCIS diagnosed on core biopsy, along with the subsequent surgical resection, from the surgical pathology files of Magee Women's Hospital. The cases were diagnosed from 2002-2007. Pathology slides and medical records for all cases were reviewed. Histologically, the PLCIS showed dyscohesive cells with grade 3 nuclei, prominent nucleoli, and moderate to abundant eosinophilic or vacuolated cytoplasm. The histological features and immunohistochemical staining for E-cadherin, P120 catenin, a basal phenotype panel (CK5, CK14, CK 17, EGFR), ER, PR, Her2/neu, and Ki-67 were evaluated in all cases.

Results: The age range of patients (pts) was 45-77 yrs, mean 61.4 yrs. Nine pts underwent core needle biopsy for calcifications and one pt for a mass lesion. All pts subsequently underwent excisional procedures (1 excision biopsy, 1 lumpectomy, 6 segmental mastectomies, and 2 simple mastectomies). Residual PLCIS was found in 100% (10/10) of excisional procedures. Invasive lobular carcinoma was found in 30% (3/10) of cases. All cases were negative for E-cadherin and showed cytoplasmic-dominant immunostaining with P120 catenin. PLCIS was positive for ER in 80% (8/10), PR in 60% (6/10), Her2-neu in 20% (2/10), and Ki-67 in 90% (9/10) of cases. Sixty percent (6/10) of cases showed some markers that may be seen in the basal-like phenotype. EGFR was negative in 100% (10/10) of cases.

Conclusions: 1. PLCIS has a lobular immunostaining pattern for P120 catenin and E-cadherin indicating disruption of the e-cadherin/P120 catenin complex. 2. PLCIS, although often ER+/PR+, has other aggressive parameters including morphology, high Ki-67 index, Her2/neu positivity and basal-like keratins. 3. PLCIS has a significant association with invasive lobular carcinoma.

159 In Situ and Invasive Carcinoma of the Female Breast Pre- and Post-Mammography

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Background: Mammography has altered our concept of the natural history of breast cancer, especially for ductal carcinoma. Screening has increased the incidence of in-situ ductal carcinoma (DCIS), but there is minimal information on in situ lobular carcinoma (LCIS). We, therefore, compared the effect of screening on both lobular and ductal in situ carcinoma.

Design: Data were retrieved from NCI's SEER Program. All racial groups were included. Temporal trends were used as a surrogate measure for screening effects. Data were stratified for pre-mammographic (1973-1982) and post-mammography (1990-2003) time periods. Age-adjusted rates (2000 U.S. standard) were expressed as cases per 100,000 woman-years. Age 50 years was our surrogate measure for menopause.

Results: SEER yielded 565,945 breast cancer cases (in situ and invasive) from 1973-2003. There were 47,523 (8.3%) DCIS and 13,229 (2.3%) LCIS tumors. Overall age-adjusted rates for DCIS were greater than LCIS, during the pre-mammographic (2.59 vs. 1.52), and post-mammographic (13.28 vs. 3.46) time periods. Premenopausal age-specific incidence rates for DCIS and LCIS were similar in 1973-1982; but were greater for DCIS than LCIS in 1990-2003. Postmenopausal rates for LCIS declined rapidly for both study periods. Postmenopausal rates for DCIS rose in the post- but not in the pre-mammographic time period.

Conclusions: Premenopausal age-specific rate patterns of DCIS and LCIS were similar before screening, suggesting similar etiology for early-onset DCIS and LCIS. This premenopausal pattern was modified by screening with the rate of DCIS increasing faster than LCIS after 1990. The overall rate of DCIS substantially increased in 1990-2003 and the age-specific rate pattern changed.

160 Dissociation between Progesterone Receptor and Cell Proliferation in Invasive Breast Cancer

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Background: The presence of estrogen receptor (ER) in breast cancer is now accepted as a predictor of extended disease-free survival, but the relative value of progesterone receptors (PR) for this purpose has not been established yet. Pichon and colleagues investigated the relationship between PR and prognosis in a small group of breast cancer patients and they concluded that the presence of PR in primary tumors were associated with a markedly lower frequency of metastasis. In this study, we analyzed the correlation between PR and cell proliferation rate to find out the association between PR expression and cell kinetics.

Design: This study included 90 patients with invasive breast cancer treated at Loyola University Medical Center during the period 2004-2006. Immunostains for PR and Ki67 were performed utilizing standard immunohistochemistry protocol. The antibodies used are as follows: PR (Ventanna Laboratory) and Ki67 (Zymed Laboratory). PR expression was assessed using Allred scoring system. The Ki67 staining pattern was assessed as favorable (<10%), borderline (10-20%) and unfavorable (>20%). The Pearson Chi-square statistical test was used for assessing significance.

Results: Of the 90 invasive breast carcinomas, 60 were PR positive (Allred score of 3 and above) and 30 were PR negative (Allred sore of 0-2). Ki67 cell proliferation rate was favorable in 22 patients, borderline in 50 patients and unfavorable in 18 patients. Of the 30 PR negative patients, 2 (6.7%) showed favorable Ki67 index, 16 (53.3%) borderline Ki67 index and 12 (40%) with high Ki67 index. There was a statistically significant inverse correlation between PR expression by Allred scoring system and cell proliferation rate by Ki67 index with a p-value of $p<0.001$.

Conclusions: This analysis demonstrates the dissociation between PR expression by Allred scoring system and cell proliferation rate by Ki67 index. PR is synthesized by cells that are stimulated by estrogen through its interaction with ER. The presence of PR reflects a functional ER pathway and therefore the presence of PR might be a better indicator of hormone dependence than ER. In conclusion, when accurately measured with proper validation, PR expression is an independent predictive factor due to its effect on cell kinetics and its expression confirms a functional ER in breast epithelial cells.

161 Phenotypic Profile of Myoepithelial Cells (MEC) in Benign Sclerosing Lesions and Ductal Carcinoma In Situ (DCIS): Diagnostic Implications

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Background: Immunostaining for MEC is commonly used to help distinguish benign and in situ from invasive breast lesions. We previously found that DCIS-associated MEC frequently show decreased expression of smooth muscle myosin heavy chain (SMMHC), limiting the utility of SMMHC as a MEC marker in this setting. Another situation in which MEC immunostaining may be useful is in the distinction of benign sclerosing lesions (BSL) from invasive carcinoma. Whether phenotypic alterations similar to those seen in DCIS-associated MEC occur in the MEC associated with BSL has not been previously studied.

Design: We performed MEC immunostains on paraffin sections of 23 BSL [15 radial scars/complex sclerosing lesions (RS/CSL) and 8 sclerosing adenosis (SA)] and, for comparison, 101 DCIS using antibodies to 7 MEC markers: smooth muscle actin (SMA), calponin (calp), SMMHC, p63, CD10, cytokeratin (CK)5/6 and p75. For each marker, the staining intensity of the MEC surrounding the entrapped glands in the BSL and around spaces involved by DCIS was compared with internal positive controls consisting of normal ducts and lobules on the same section.

Results: MEC in BSL and DCIS showed phenotypic differences from normal MEC and from each other, particularly with regard to expression of CK5/6 and SMMHC (see Table). Sixty-two percent of BSL showed reduced MEC expression of CK5/6 compared to 31% of DCIS (p=0.009). In contrast, MEC SMMHC expression was reduced in 78% of cases of DCIS compared to 37% of BSL (p=0.0006).

Table. Proportion of Cases of BSL and DCIS with Reduced Expression of MEC Markers

	SMA	Calp	SMMHC	p63	CD10	CK 5/6	p75
BSL (n=23)	0%	14%	37%	10%	19%	62%	0%
DCIS (n=101)	1%	16%	78%	8%	30%	31%	5%
p-value	NS	NS	0.0006	NS	NS	0.009	NS

Conclusions: MEC associated with BSL exhibit a phenotypic profile that differs from that of both normal and DCIS-associated MEC. In particular, expression of CK5/6 is more often absent or reduced in the MEC of BSL than in the MEC associated with DCIS whereas decreased SMMHC expression is more frequent in DCIS than in BSL. The practical implication of these observations is that the differential diagnostic question being posed (e.g., invasion vs DCIS or invasion vs benign sclerosing lesion) should influence the choice of MEC markers chosen for diagnostic use since the sensitivity of some of these markers appears to differ in different pathologic processes.

162 Mammary Adenoid Cystic Carcinoma: A Biologically *Triphasic* Neoplasm of Luminal, Myoepithelial and Non-Committed CK14+, p63+ Basal Cells

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Background: Primary mammary adenoid cystic carcinoma (MACC) is a rare neoplasm popularly considered to be a biphasic tumour of luminal and myoepithelial cells. The recent availability of an increasing range of immunomarkers has allowed more in-depth and precise characterization of tumours. Using immunohistochemistry, we aimed to elucidate the biological profile of MACC.

Design: 8 cases of MACC were studied using conventional H&E and also evaluated for their expression of the following immunomarkers: epithelial membrane antigen (EMA), cytokeratins CK5/6 and CK14, smooth muscle actin (SMA), smooth muscle myosin-heavy chain (SMM), and p63, using the avidin biotin methodology. A semi-quantitative assessment of the proportion of tumour cells staining for the individual marker was performed.

Results: On H&E, all MACCs showed cribriform-tubular architectures, with solid areas comprising less than 30% of the entire tumour. All tumour cells displayed basaloid features, yet it was possible to distinguish a small dispersed proportion of luminal cells, which formed ductal structures, from the majority of non-luminal cells which swirled around pseudoluminal spaces. EMA was positive in the luminal cells only. A similar profile was observed with CK5/6, although the latter was also detected occasionally in cells that were oriented around pseudoluminal spaces in 1 of 8 cases. CK14 was diffusely positive in all MACCs (75% to 100% of tumour cells), highlighting both luminal and non-luminal cells. All tumour cells were negative for SMM. p63 was expressed by virtually all non-luminal cells and not by luminal cells. Similarly, none of the luminal cells were identified with SMA. Among the non-luminal cells, there was variable positivity (5 to 80%) for SMA, thus identifying a subset of non-myoepithelial (SMA-,SMM-), non-luminal (EMA-), p63+, CK14+ tumour cells.

Conclusions: Contrary to conventional wisdom, MACC is a biologically *triphasic* neoplasm composed not only of luminal and myoepithelial cells, but also of a third population of hitherto poorly understood non-committed basal cells indistinguishable from myoepithelial cells on pure morphologic grounds. Of interest, these basal cells and myoepithelial cells appear to constitute the vast majority of the tumour cell population in MACCs. CK14 and p63 immunostains are diagnostically useful adjuncts in the distinction of MACCs from cribriform ductal carcinomas.

163 New Cutoff Points of Tumor Size Discriminates Patients' Survival Time More Precisely Than T Classification of the 6th AJCC Cancer Staging System of Breast Carcinoma

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Background: Tumor (T) classification is one of the most important components of TNM system, and provides information regarding prognosis and treatment options for patients with breast carcinomas. Therefore, in order to estimate more precise outcome of patients, application of the more refined staging system is necessary.

Design: We evaluated tumor size in 609 patients of breast carcinoma by measuring only infiltrating breast carcinoma component, and compared this evaluation to survival time and other clinicopathologic parameters, including the current T classification of AJCC cancer staging system.

Results: A complex pattern of survival time versus the tumor size was observed by censored local regression. The recursive-partitioning technique was coupled with the log-rank test to identify 2 significant cutoff points for the tumor size, 3.2 cm and 5 cm, which segregated patients into 3 groups with statistically significant decreasing 5 year survival rates (<3.2cm, 95.5%; 3.2-5 cm, 88.5%; >5 cm, 65%, $p<0.001$), which discriminate outcome of patients with breast carcinoma much better than those with the current 6th T classification of AJCC cancer staging system (T_1 classification, ≤2 cm, 97.2%; T_2, >2cm and <5 cm, 92.7%; T_3, > 5 cm, 65%).

Conclusions: Based on the present data, we propose that the T classification of breast carcinoma should be changed to incorporate this measurement: T_1 (<3.2 cm), T_2 (3.2-5 cm), and T_3 (>5 cm).

164 Novel Approaches to Breast Tissue Banking: The Duke Breast SPORE Experience

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Background: Due to the efficacy of neoadjuvant chemotherapy and earlier detection, many neoplastic lesions of the breast are grossly inapparent or too small for typical tissue procurement protocols. We describe a new method to sample breast lumpectomy specimens and also provide data to document that pathologic verification of banked breast tissues is critical.

Design: This study includes needle core biopsies of benign and malignant breast tissues included in the Duke Breast SPORE Tissue Bank. 36.5% of the cores were obtained from patients undergoing radiologically guided biopsy of suspicious breast lesions (average 1-4 cores per case). 63.5% of the cores were obtained from lumpectomy specimens (average 2-6 cores per case). The latter procedure utilized a new technique whereby a lesion is radiographically localized, immobilized in a holding device, and sampled via a spring-loaded needle biopsy gun.

Results: Review of frozen sections prepared from 3262 cores from 896 patients revealed concordance with the pathologic diagnosis in 1964 (60.2%) and discordance in 1298 (39.8%) cores. Among the 2073 cores derived from lumpectomy specimens, 1125 (54.3%) were concordant, and 948 (45.7%) were discordant with the specimen diagnosis. Among the 1189 cores obtained in Radiology, 839 (70.6%) were concordant, and 350 (29.4%) were discordant with the surgical pathology diagnosis. On a per case basis,

487 cases (54.4%) were completely concordant, while 215 (24.0%) were partially, and 194 (21.7%) completely discordant. In almost all cases, discordance reflected absence of the index lesion in the research cores. In only four cases (<0.5%) one of the research cores contained significant pathology that was not present in the diagnostic material. The banked cores included a relatively high number of DCIS lesions.
Conclusions: This study shows that it is possible to bank fresh frozen tissue from small and grossly inapparent breast lesions without compromising surgical margins or diagnostic evaluation. In addition, our technique allows sampling of more precursor lesions and small tumors than conventional methods. The concordance rate in lumpectomies sampled by our technique is higher than that expected under visual or palpation guidance. Nevertheless, between 30 and 45% of banked cores do not represent the index lesion of interest, suggesting that pathologic verification of banked breast tissue is critical before such samples are used in research studies.

165 Somatic Mutations in Steroid Hormone Receptor Coactivators in Breast Cancer

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Background: Steroid receptors interact with a series of coactivators to stimulate transcriptional activation of specific target genes. One such coactivator family is referred to as the p160 family and includes steroid receptor coactivator 1 (SRC1), glucocorticoid receptor interacting protein 1 (GRIP1/SRC2) and amplified in breast cancer 1 (AIB1/SRC3). Related to AIB1 and located close to it, on chromosome 20, is another nuclear receptor coactivator, amplified in breast cancer 3 (AIB3). Both AIB1 and AIB3 are amplified and overexpressed in breast cancer. Although single nucleotide polymorphisms (SNPs) are well characterized in the coactivator genes, little is known about somatic mutations of the steroid hormone receptor coactivators.
Design: Genomic DNA was isolated from 11 breast cancer cell lines, one normal immortalized breast epithelial cell line and 82 breast cancer specimens. The tumor samples were collected from patients of four ethnicities: African-American, Latina, Asian and Caucasian. Cell line DNA was assessed for mutations in the SRC1, GRIP1, AIB1 and AIB3 genes by fluorescent sequencing. Tumor DNA was also sequenced for AIB3 in order to confirm the frequency of mutations identified in the cell lines in this gene.
Results: Genetic alterations were characterized in the p160 family of proteins (SRC1, GRIP1, and AIB1). A total of seven known SNPs and one new synonymous mutation were identified in these genes. No new non-synonymous mutations were identified in the cell line DNA, therefore no tumor DNA was sequenced for these genes. In contrast, two novel non-synonymous mutations were identified in cell line DNA for AIB3, one each in SKBR3 and MDA-MB-435. Four additional non-synonymous mutations were assessed by the evaluation of AIB3 in 82 breast cancer specimens. Each of these mutations was identified in one breast tumor. In addition, there were six new silent mutations and four known SNPs identified in the AIB3 gene.
Conclusions: Six new AIB3 non-synonymous sequence alterations were identified within the C-terminal region of the gene or close to it. The C-terminal domain, containing four of these mutations, is known to interact with DNA dependent protein kinase and with other coactivator proteins. Therefore, modifications in this region may alter the function of AIB3. This could affect steroid hormone receptor activity and subsequently influence the aggressiveness, metastatic capacity or response to therapeutic agents of breast tumor cells.

166 Morphologic and Clinical Characterization of "Triple-Positive" Invasive Breast Carcinomas

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Background: Based on recent gene profiling studies, it is now known that invasive breast cancer is a group of heterogeneous diseases broadly divided into the luminal (ER+) and basal (ER/PR and Her2 negative; triple negative) subgroups. The luminal group has been further subdivided into types A,B, and C with type A having the best prognosis and type C the worst, also characterized by being Her2 positive. While the morphologic, immunohistochemical and clinical characteristics of the basal subgroup (triple negative) has been well characterized, little has been done with the luminal subtype. Our goal was to specifically study the clinical and morphologic features of the luminal C subtype (triple positive).
Design: Using the pathology immunohistochemistry database from 1/2000 to 6/2007, we retrospectively identified 90 cases of invasive mammary carcinomas with positive ER/PR (<5-100%) and Her2Neu (3+,>50%). Slides for review of the carcinoma were available in 70 cases. Clinical and histopathologic information such as patient age, tumor size, extent of DCIS and lymph node status was gathered.
Results: The age of the patients ranged from 35 to 84 years (average=57). The size of the carcinomas ranged from microinvasive to 6.5cm (average=1.6cm). They were histologically characterized as ductal=54 cases, lobular=5 and mixed=11. By differentiation, the pure and mixed ductal cases were classified as follows: well=4 cases, moderately=24, poorly=37 and by morphology as follows: micropapillary = 44 cases, tubular and cribriform =5 and typical = 16. The micropapillary components ranged from being focal (15 cases) to partial (18) to predominant (11). The lobular components in the pure and mixed category were as follows: tubulo-lobular=4, pleomorphic=10, typical =2. Intraductal carcinoma was present in 61 cases, extensive in 19 cases and by grade divided as follows: low=2, intermediate= 24 and high=35. Axillary lymph nodes were involved by metastatic carcinoma in 28 cases, most of which were partially replaced.
Conclusions: The triple positive tumors represent a subset of breast carcinomas that occur more commonly in postmenopausal women, are predominantly moderately to poorly differentiated duct carcinomas with some extent of micropapillary differentiation (63%). When a lobular component was present, it was predominantly pleomorphic (63%). Both phenotypes represent aggressive variants of breast carcinoma. Clinical and histopathological characterization of these tumors will assist in their identification and assist in the development of optimal targeted treatment strategies.

167 HER2 Status in a Large, Population-Based Cohort: Analysis of Distinct HER2 Subgroups

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Background: HER2 gene amplification and/or protein overexpression in breast cancer is associated with a poor prognosis and predicts response to anti-HER2 therapy. We examine the natural history of breast cancers in relationship to HER2 amplification and expression in a large population-based study.
Design: HER2 status was measured by fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) in approximately 1400 breast cancer cases with greater than 15 years of follow-up. Protein expression was evaluated with two different commercially-available antibodies.
Results: Using survival data, we looked for natural breast cancer groupings based on HER2 FISH amplification ratio. The current HER2 ratio cut point for classifying HER2 positive and negative cases is 2.2. However, we found that HER2 ratio cut points of 1.0 and 1.5 delineate breast cancers by distinct risk groups, with increased risk associated with amplification ratios of greater than 1.5. An intermediate group of cases with HER2 amplification ratios between 1.5 and 2.2 was found to have a significantly better outcome than the conventional amplified group (HER2 ratio greater than 2.2) but a significantly worse outcome than groups with amplification ratios less than 1.5.
Conclusions: Breast cancers with low level HER2 amplification, below the currently accepted positive threshold ratio of 2.2, showed a distinct, intermediate outcome when compared to HER2 unamplified tumors and tumors with HER2 ratios greater than 2.2. These findings suggest that a new cut point to determine HER2 positivity, at a ratio of 1.5 (well below the current recommended cut point of 2.2), should be evaluated.

168 FHIT and WWOX Expression Correlates with Hormone Receptor Status: A Tissue Microarray Study of 240 Breast Carcinomas

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Background: FHIT and WWOX are tumor suppressor genes whose reduced or absent expression has been associated with diverse prognostic factors in breast cancer. It has been noted that they are inactivated coordinately in invasive breast carcinoma. Inactivation of WWOX has been previously related to negative hormone receptor status, as well as tamoxifen resistance in estrogen receptor (ER) positive cases. We assessed the relationship between these markers and traditional prognostic markers in a large cohort of breast cancers.
Design: Tissue microarrays from 240 breast carcinomas were built and resulting sections stained with anti Fhit, Wwox, ER, progesterone receptor (PR), and Her-2/neu. For Fhit and Wwox, status was considered negative if <5% of cells showed cytoplasmic staining. For ER and PR, status was considered positive if >1% of cells showed nuclear staining. For Her-2/neu, membranous staining was scored in the usual fashion as 0, 1+, 2+ or 3+, according to intensity and extent, and considered positive if 2+ or higher.
Results: Mean age of patients included was 52.6, median 53 (104 cases were <50 years old). Cytoplasmic staining for Fhit was observed in 156/236 cases (66.1%), and for Wwox in 168/235 cases (71.49%). 171 (71.3%) and 135 (56.3%) of 240 cases were positive for ER and PR, respectively. Her-2/neu was overexpressed in 65/237 cases (27.4%). 122 of 156 (78.2%) Fhit positive cases were ER positive, compared to 46 of 80 (57.5%) Fhit negative cases (p=0.0013). 140 of 168 (83.3%) Wwox positive cases were ER positive, compared to 30 of 67 (44.7%) Wwox negative cases (p<0.0001). Multivariate analysis by binary logistic regression showed that Wwox and PR correlate with ER (prediction rate 86.9%). ER and Fhit correlate with PR (prediction rate of 84.7%). Wwox, Fhit, PR, and age of diagnosis are all needed to predict Her-2/neu status (prediction rate of 77.3%).
Conclusions: Our data support that Fhit and Wwox are good predictors of hormone receptors status, and are less strongly correlated with Her-2/neu over-expression. Understanding the mechanisms involved in the molecular interactions among these proteins and their signal pathways may reveal new therapeutic targets that could potentially impact hormonal treatment of breast cancer.

169 Expression of Na+/I- Symporter Transmembrane Protein in Malignant and Benign Breast Epithelium: A Tissue Microarray Study

RE Jimenez, SJ Beyer, L Gentchev, SM Jhiang. The Ohio State University, Columbus, OH.
Background: The Na+/I- symporter (NIS) is a transmembrane protein responsible for the uptake of iodide in thyroid follicular cells. This protein has also been detected in up to 80% of breast cancers and in fibroadenomas. There is considerable interest in factors that can selectively up-regulate NIS function in breast tumors as they may facilitate the use of radionuclide imaging for detection and treatment of primary, residual or metastatic breast cancer. It is not clear whether increased expression of NIS occurs only on the tumor epithelium or is also present in the surrounding normal breast tissue.
Design: Tissue microarrays containing samples from 210 breast carcinomas and corresponding adjacent normal tissue were built and stained with NIS p442 antibody (Sigma Genosys, The Woodlands, TX). An additional TMA containing samples from 54 fibroadenomas was built and also stained with NIS p442. Only membranous staining was considered for scoring. Cases were scored with a scale of 0, 1+, 2+, and 3+, using similar criteria as those used for Her-2/neu staining scoring. Cases with staining patterns 0 or 1+ were considered negative, and those with 2+ or 3+ staining were considered positive.
Results: Of the 192 evaluable carcinomas, 55 (28.6%) showed positive staining (40 score 2+, 15 score 3+), while 137 showed negative staining (54 score 0, 83 score 1+). In 149 cases there was corresponding readable normal tissue, 5 of which (3.4%) showed

positive staining of the non-neoplastic component ($p<0.0001$). Four of 45 tumors (8.9%) with positive staining showed also positive staining in the normal component, while only 1 of 104 tumors (1.0%) with negative staining showed positive staining in the normal component ($p=0.0292$). Of 38 evaluable fibroadenomas, 34 (89.5%) showed staining 1+ or stronger, 3 of them (8%) 2+ or stronger.

Conclusions: NIS is expressed in approximately 30% of breast carcinomas, but strong expression in normal breast tissue is relatively uncommon. While weak staining is seen in most fibroadenomas, the intensity of expression is considerably lower than the one observed in malignant tumors. The current data suggest that tumor microenvironment may play an important role on the upregulation of NIS in breast cancer. Understanding of the factors that can selectively up-regulate NIS function in breast cancer could result in data that may impact diagnosis and treatment of breast cancer.

170 Dual Expression of a-Tocopherol Associated Protein (TAP) and ER in Normal Human Breast Epithelium and Its Down Regulation in ER Positive Carcinomas

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Background: Breast carcinogenesis involves hormonal-driven cell proliferation and genetic alterations, including oncogene activation and suppressor gene down regulation. Our previous study showed that TAP is down regulated in breast cancer cell lines and human breast carcinomas, indicating its tumor suppressor-like function. Correlating TAP expression with ER will further signify its role in breast carcinogenesis.

Design: 20 breast core biopsies, 10 from premenopausal patients and 10 from postmenopausal patients, were identified. 10 cases (5 from each group) are with normal/benign breast epithelium, the other 10 with invasive carcinoma and adjacent normal/benign breast epithelium. Immunohistochemical stain for TAP and ER was performed on consecutive sections with 3.0 um thickness. 141 breast excisional specimens with invasive carcinoma were identified. Immunohistochemical stain for TAP and ER was performed on representative sections with invasive carcinoma and adjacent normal breast tissue. Fisher's exact test was used for statistic analysis.

Results: The normal/benign breast epithelium is universally scattered positive for ER and TAP. In a given terminal ductal lobular unit on the consecutive sections, dual exrpession of ER and TAP is observed in the individual epithelial cells. This observation is true regardless of the menopausal status or from cases with or without invasive breast carcinoma. TAP is down regulated in 80 of 141 (57%) invasive carcinomas (table 1), 40% of non-high grade, 80% of high grade, similar trend as our previous preliminary findings.

TAP expression in human breast carcinomas

	TAP +	TAP -	total
High grade	12	47	59
Non-high grade	49	33	82
total	61	80	141

In 138 cases with ER/PR expression information, 53 of 108 (49%) ER/PR positive carcinomas are TAP negative (Table 2).

Correlating the expression of TAP with ER/PR

	TAP +	TAP -	total
ER/PR +	55	53	108
ER/PR -	6	24	30
total	61	77	138

Conclusions: TAP is down regulated in breast carcinomas, especially in those of high grade. TAP is co-expressed with ER in normal/benign breast epithelium cells, regardless of patient age or history of breast cancer. Nevertheless, TAP is negative in 49% of ER positive invasive carcinomas. This finding indicates that in the hormonal carcinogenesis, TAP, as a tumor suppressor-like molecule, may have helped to maintain the normal/benign breast epithelium during hormonal-driven cell proliferation and its down regulation may have triggered the process of hormonal carcinogenesis.

171 Evaluation of the Anterior Margins in Skin-Sparing Mastectomies for Breast Carcinoma: Pathologic Findings and Their Clinical Implications

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Background: Skin-sparing mastectomy (SSM) with immediate reconstruction is increasingly performed for the treatment of breast carcinoma (BC) to improve cosmesis. The assessment of the anterior margin (AM) of the SSM is not routinely performed at all centers. However, one concern is that not all of the BC is removed at the AM of the SSM, where glandular breast parenchyma interminges with the dermis of the skin flaps, potentially promoting local recurrences. The objective of this study is to determine the frequency of finding carcinoma at or close to the AM.

Design: We analyzed 50 SSMs (10 ductal carcinoma in-situ (DCIS), 2 lobular carcinoma in-situ (LCIS) and 38 invasive carcinoma), which were performed by a fellowship-trained breast surgeon. We also analyzed re-excised AMs (RAM), which often involved the overlying skin on cases with positive or close AMs. SSMs were evaluated for AM status (distance of BC from the inked AM and the number of foci of BC within 1 mm of the inked AM). A margin was considered positive if BC was at or within 1 mm of the inked surface, and close if BC was present between 1 to 5 mm of the inked margin.

Results: Fifteen of 50 SSMs (30%) had a positive AM and 12 of 50 (17%) SSMs had a close AM. Three of 14 (21%) RAM had residual disease. Factors predictive of a positive AM included presence of multiple foci of BC, extent of the tumor and a higher number of quadrants involved by BC.

Conclusions: The AMs in SSMs are often positive. Approximately one third of RAMs contain residual carcinoma, likely reflecting the difficulty in completely removing neoplastic and non-neoplastic breast tissue near the skin flaps in SSMs. Therefore, AM sampling is important. Re-excision of positive AMs may decrease the chance of local recurrence.

172 Comparison of Automated Silver Enhanced In Situ Hybridization (SISH) and Fluorescent In Situ Hybridization (FISH) for Assessment of Her2/neu Gene Status in Invasive Breast Carcinomas

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Background: To date assessment of Her2/neu status is important in the standard of care in the management of breast cancer. Although evaluation of Her2/neu gene amplification by FISH has excellent sensitivity and specificity, it requires special skills and equipment and thus is not performed by many labs. Recently SISH methodology which combinds bright field microscopy and automation has become available. The aim of this study was to compare Her2/neu gene amplification by both methods in a series of invasive breast carcinomas.

Design: Her2/neu gene amplification was assessed in 101 cases of invasive breast carcinoma by FISH (Vysis Pathvision) and by SISH (Ventana Ultraview SISH kit). The tumors were assessed using the ASCO/CAP and Canadian guidelines. A tumor was positive for Her2/neu gene amplification if the ratio of Her2/neu gene signals to chromosome 17 signals was > than 2.2. The tumor was negative for Her2/neu gene amplification if the ratio was <1.8. The tumor was considered as equivocal for Her2/neu gene amplification if the ratio was between 1.8 and 2.2.

Results: This study showed that 99/101 (>98%) of cases were concordant for FISH and SISH. 2/101 cases were discordant. In one case FISH was negative whereas SISH was equivocal. In the other case FISH was equivocal whereas SISH was not amplified (Table 1).

Table 1

	FISH	SISH	Comment
Amplified	41	41	100% concordance
Not amplified	55	55	(1 case discordant*)
Equivocal	5	5	(1 case discordant**)
	101	101	
2 cases with discordance:			
* 1 case FISH - not amplified, SISH - equivocal			
** 1 case FISH - equivocal, SISH - not amplified			

Conclusions: The 98% concordance between FISH and SISH meets the ASCO/CAP requirements for test validation of >95% concordance for amplified versus non-amplified cases. These results indicate that SISH can be used as an alternative to FISH in the assessment of HER2/neu gene status in breast carcinomas.

173 Podoplanin Expression in Myoepithelial Cells and Basal Cells in Breast and Prostate Tissue; Utility and Potential Pitfalls

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Background: Podoplanin is a mucin-type transmembrane glycoprotein originally tauted as a specific and sensitive marker for normal lymphatic channels. It has been suggested that podoplanin is useful to assess for angiolymphatic invasion in histologically controversial cases of breast cancer. However, rare reports have shown that nonlymphatic benign supporting cells from a variety of tisuue sites are immunoreactive for podoplanin. This study was designed to 1. assess the staining pattern of podoplanin in breast tissue and 2. to evaluate podoplanin reactivity in the basal cells in prostate glands.

Design: Archival paraffin-embedded tissue blocks of 12 breast carcinomas (7 micropapillary, 5 grade 2 ductal carcinomas) and 8 blocks of prostatic adenocarcinoma with benign and malignant glands (Gleason scores 6-9) were collected. All cases were stained with Podoplanin (1:200, monoclonal antibody, antimouse, Angiobio Co.) using the Dako autostainer. Each breast case was assessed for podoplanin staining of lymphatics, cancer, and myoepithelial cells around normal ducts and DCIS. Each prostate case was assessed for and the distribution of podoplanin expression. Cytoplasmic staining was scored as 0, 1 or 2 (0= no staining, 1= weak, 2= strong).

Results: Breast Carcinomas: Podoplanin uniformly stained lymphatics strongly (2+). The myoepithelial cells in the normal breast ducts and around the in situ carcinomas was uniformly positive but varied in intensity from 1-2+. None of the breast carcinomas were immunoreactive for podoplanin. Prostate Carcinomas: The basal cells in the normal prostatic glands were 1-2+ in all cases. The basal cell staining was diffusely positive but variably intense. The prostate carcinomas were negative for podoplanin.

Conclusions: * Podoplanin highlights myoepithelial cells in breast tissue. * Podoplanin is helpful in distinguishing micropapillary pattern of ductal carcinoma from angiolymphatic invasion. * DCIS with retraction artifact can be mistaken for angiolymphatic invasion. Podoplanin to detect lymphatic invasion must be interpreted with care to avoid this pitfall. * Basal cells in prostate strongly react with podoplanin and can be used as a basal cell immunohistochemical stain.

174 Clinico-Histopathological Correlation of Magnetic Resonance Image Guided Breast Core Biopsy: Institutional Review of Our Initial Experience

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Background: Magnetic Resonance Imaging (MRI) is a highly sensitive test for detecting infiltrating ductal carcinoma and, to a lesser extent, ductal carcinoma in situ (DCIS) especially in high risk women. MRI can detect malignancy by vascular enhancement, often in cases lacking mammographic and ultrasound abnormalities. MRI-guided tissue sampling can be accomplished either by wire localization with surgical excision or by core biopsy. Core biopsy has several advantages over wire localization such as decreased invasiveness, morbidity, and cost. At our institution a correlative (second-look) ultrasound is performed for all lesions seen with MRI. If a sonographic correlate is identified, biopsy is performed under ultrasound-guidance. Only for those lesions for which an ultrasound correlate cannot be identified is an MRI-guided biopsy performed. The objective of this study was to evaluate our initial experience with MRI-guided core breast biopsy.

Design: A retrospective review over a period of 36 months revealed 107 MRI-guided core biopsies performed for MRI-detected breast lesions in a high risk population. The median age of the patients was 47 years (range: 29–77). Histology of all the lesions was obtained and surgical follow-ups were reviewed when available.
Results: Of the107 MRI guided core biopsies, 100 (93%) showed benign histology encompassing a spectrum of benign entities. Follow-up with surgical excision with wire localization was performed in 4 of the benign entities showing 2 intraductal papillomas, 1 fibroadenoma, and 1 cellular fibroepithelial lesion. Malignancy was identified in 4 (4%) of 107 cases: 3 infiltrating ductal carcinomas and 1 DCIS. Surgical excision of the biopsies positive for malignancy showed infiltrating ductal carcinoma in all four cases. Three (3%) of 107 cases were pre-malignant: atypical ductal hyperplasia (ADH) was present in 2 biopsies and 1 case was diagnosed as lobular neoplasia (LN). Surgical follow-up of these cases showed benign breast.
Conclusions: Carcinoma can be detected in MRI of the breast as a vascular enhancement, even in the absence of clinical, mammographic or ultrasound findings. Imaging-histologic correlation is essential to ensure lesion sampling.

175 Comparison of Estrogen Receptor, Progesterone Receptor and Her-2 Status in Breast Cancer Pre- and Post-Neoadjuvant Chemotherapy

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Background: Neoadjuvant chemotherapy (NAC) is now a relatively standard treatment for breast carcinoma. However, some tumors are known to develop resistance to chemotherapies after an initial period of sensitivity.
Design: We investigated whether the status of estrogen receptor (ER), progesterone receptor (PR) and Her-2 expressions could be changed in breast cancer cases following NAC. We retrospectively examined 173 cases from 212 breast cancer patients with NAC from 2002 to 2007. In these 173 cases, we analyzed routine reports between core biopsies prior to NAC and surgical specimens after NAC, with regard to expressions of ER, PR, Her-2, histologic grade, and response. Additionally, 117 non-NAC controls which had core biopsies prior to surgery and surgical specimens available, were examined from the same time period. We used immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods.
Results: No differences were found in ER or Her-2 status pre- or post- NAC, but a statistically significant difference was found in PR status, which was predominantly changed from positive to negative following NAC. Several cases in which Her-2 status changed after NAC, as indicated by IHC, showed no changes by FISH. ER changes were related to good histologic response and PR changes were associated with low to intermediate grade carcinomas.
Conclusions: As ER changes were not significant after NAC, loss of PR might not be predictive for subsequent hormone therapy. For patients receiving NAC, it may not be necessary to repeat testing for ER, PR, and Her-2 expression. When IHC indicates a change in Her-2 expression, FISH is recommended.

Table 1 Summery of changes in case and control groups

	Case	Control	Mann-Whiteney Test
ER	19/173(11.0%)	8/117(6.8%)	P=0.234
PR	27/173(15.6%)	9/117(7.7%)	P=0.045
Her-2	0/173(0%)	0/117(0%)	P=1

ER, estrogen receptor; PR, progesterone receptor

Table 2 Summary of the results: cases

	pos→posi	neg→neg	neg→pos	pos→neg
ER	87/173(50.3%)	67/173(38.7%)	17/173(9.8%)	2/173(1.2%)
PR	47/173(27.2%)	99/173(57.2%)	8/173(4.6%)	19/173(11.0%)
Her-2	40/173(23.1%)	135/173(76.9%)	0	0

ER, estrogen receptor; PR, progesterone receptor; pos, positive; neg, negative

176 Novel Biologic Markers in Phyllodes Tumors

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Background: Phyllodes tumors (PT) represent 2.5% of breast fibroepithelial tumors, with highly variable malignant potential. The WHO-2003 subdivides PT into three categories: benign, borderline, and malignant, based on histopathologic characteristics; however diagnostic criteria are imprecise. Few small studies have applied different biological markers (p53, c-kit/CD117, c-Erb-2, Ki-67), to facilitate classification of PT. A recent study described a malignant PT with amplification of MDM2 locus (12q13-15, murine double minute2 homolog, regulates p53; Mod Pathol. 20:435-44). Overall, the results from these studies have been inconsistent, with a trend toward increased Ki-67 proliferation index and increased p53 expression in malignant PT. We sought to correlate histologic grade with differences in expression of these and other novel markers in phyllodes tumors.
Design: Formalin fixed, paraffin embedded sections of 14 breast fibroepithelial tumors, 12 PT (3 benign, 4 borderline, and 5 malignant) and 2 cellular fibroadenomas (cFA) were immunohistochemically stained using routine protocols, with antibodies to p53 (clone DO-7), c-kit (9.7), the M-phase marker phospho-Histone3 (polyclonal, pH3), mdm2 (1F2), cdk4 (DCS-31, genetic locus near MDM2 at 12q13-5). Stromal, myoepithelial, and epithelial cell reactivity (semiquantitative %, intensity) were scored by two pathologists and subsequently correlated with histopathologic grade.
Results: Stromal p53 expression was significantly greater in malignant PT compared to all other groups combined (p=0.01 nonparametric Mann-Whitney U, Table 1). pH3 demonstrated a trend toward high mitotic rate in malignant PT, and an elevated rate in borderline PT (Table1), but was noted to be especially sensitive to proper formalin fixation. No significant difference in stromal or epithelial expression of c-kit between subgroups of PTs was identified; cdk4 and mdm2 expression was not detected in any samples.

Table 1

	Malignant PT	Borderline PT	Benign PT	cFA
Stromal p53(%+/-SEM)	71.3 +/-14.8	26.3 +/-8.0	16.2 +/-3.9	0.7 +/-0.3
Stromal pH3 (#/10HPF+/-SEM)	22.6 +/-12.2	6.6 +/-3.9	0.8 +/-0.5	0

Conclusions: Stromal expression of p53 and pH3 were consistently high in malignant PT, and elevated in borderline PT. No correlation was found between stromal/epithelial c-kit protein expression and PT histologic grade. Although a previous study suggested that 12q13-15 may be altered in phyllodes tumors, our series showed no increased expression of cdk4 or mdm2 protein.

177 Poor Prognostic Significance of Unamplified Chromosome 17 Polysomy in Invasive Breast Carcinoma

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Background: Her2 gene amplification has been established to be a poor prognostic indicator in breast carcinoma. Some patients show chromosome 17 polysomy with or without Her2 amplification. The significance of 17 polysomy without Her2 amplification is uncertain. The aim of this study was to determine the significance of 17 polysomy without Her2 amplification on prognostic and predictive indicators in invasive breast carcinoma.
Design: Polysomy 17 was defined by the presence of greater than three chromosome 17 centromere copies/cell. Three groups: **N** (no polysomy and no amplification, 36 cases), **P** (17 polysomy without Her2 amplification, 21 cases) and **A** (Her2 amplification without 17 polysomy, 24 cases) were compared for the following: Nottingham score, tubule score, nuclear grade, mitotic score, histological grade, presence of lymphovascular invasion, nodal metastases, T stage, estrogen receptor (ER) and progesterone receptor (PR) negativity. Mode was determined to be the best statistical tool, and a 5% difference between any two groups for cumulative % was statistically significant.
Results: The cumulative percentage of prognostic and predictive indicators is given in the table below.

Cumulative percentage of prognostic indicators

Prognostic indicators	N	P	A	N vs. P	P vs. A
Nottingham score-8	13.3	35.0	40.0	p < 0.005	p = 0.05
Nuclear grade-3	27.0	65.0	74.0	p < 0.005	p = 0.015
Mitotic score-2	17.9	52.6	50.0	p < 0.005	-
T stage-2	21.2	30.0	29.4	p = 0.02	-
LVI present	18.9	25.0	31.6	p = 0.04	p = 0.04
positive LN	38.0	47.0	31.6*	p = 0.04	p < 0.01*
ER negativity	11.1	14.3	45.8	-	p < 0.005
PR negativity	30.6	33.3	45.8	-	p < 0.01

- = not statistically significant; *- four cases had prior neoadjuvant therapy

Conclusions: Invasive breast carcinoma with chromosome 17 polysomy is associated with adverse prognostic indicators such as a higher Nottingham score, nuclear grade and mitotic activity similar to the amplified group, in contrast to patients with neither amplification or polysomy. For T- stage and lymphovascular invasion, the polysomy group shows a trend towards the amplified group. However, polysomy 17 does not appear to influence hormone receptor expression.

178 Correlation of Chromosome 17 Polysomy with Her2/neu Immunohistochemistry Status

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Background: Patients with amplification of Her2/neu gene demonstrated by FISH or overexpression of Her2/neu protein by immunohistochemistry (IHC) qualify for treatment by anti Her2 immunotherapy. However, some patients show chromosome 17 polysomy with or without Her2 amplification. The significance of 17 polysomy without amplification is uncertain. The aim of this study was to study the correlation between chromosome 17 polysomy and Her2/neu IHC status.
Design: A total of 160 cases analyzed for Her2 status by FISH were divided into four groups: **N** (no polysomy and no amplification), **P** (17 polysomy without amplification), **A** (amplification without polysomy) and **AP** (amplification with polysomy). The results obtained by FISH were compared with the IHC score. Cases were also analyzed for the number of Her2 copies/cell and CEP17 copies/cell.
Results: Correlation of Her2/neu FISH, IHC score and polysomy status is shown in the table below.

Correlation of Her2/neu FISH, IHC score and Polysomy status

IHC score	N	P	A	AP
0/1+ (32 cases)	84.3%	9.4%	6.3%	0%
2+ (92 cases)	74.9%	20.6%	3.5%	1.0%
3+ (36 cases)	2.7%	2.7%	69.8%	25.0%

Polysomy was seen in 20.6% of all cases (33/160), with 60.6% in the 2+ and 30.3% in the 3+ IHC groups. 80% of the patients with non amplified polysomy have a 2+ IHC score. The number of Her2 copies/cell showed a bimodal distribution in **P, A** and **AP** groups. In the **P** group it ranged from 3.1 to 6.6 with peaks at 3.6 and 5.2, from 5.4 to 25 with peaks at 10 and 18 in the **A** group, and from 10.6 to 26.2 with peaks at 5 and 20 in the **AP** group. The difference between the **P** and both the **A** and **AP** groups was statistically significant (p<0.001), but the difference between the **A** and **AP** groups was not significant. The CEP17 copies/cell in the **A** group showed a normal distribution in the range of 1.4 to 3.0. In the **P** and **AP** groups the range of CEP17 copies/cell was 3.0 to 5.4 with a single peak between 3.0-3.25, and the difference was not significant.
Conclusions: Polysomy of chromosome 17 plays a role in increased IHC expression of Her2 protein, with non amplified polysomy resulting predominantly in 2+ scores. While the absolute number of Her2/neu copies/cell is increased in the group with 17 polysomy without amplification, it is much lower than that seen in cases with Her2/neu amplification.

179 What Is the Value of a Third Re-Excision in Breast Conserving Therapy?

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Background: More than three-quarters of women with breast cancer choose breast conserving therapy (BCT) over mastectomy. Microscopically clear BCT margins can be achieved in the vast majority of cases with lumpectomy or lumpectomy plus one or two re-excisions (RE). We studied patients who chose to undergo three or more RE to determine: Overall numbers and demographics, pathologic findings necessitating re-excision, rate of mastectomy, and cosmetic result.

Design: Records of 3536 Breast Cancer Registry patients from 1/1/2000-6/30/2007 were reviewed. Patients who underwent a lumpectomy followed by three or more RE with or without mastectomy were included in the study. Those who received preoperative chemotherapy were excluded.

Results: Fourteen women met the above criteria. They ranged in age from 41-79 years (average 53 years); ten were Caucasian and four African-American. Thirteen patients had three RE, and one had four RE. Three patients ultimately received a mastectomy. Two of these were the only patients with invasive lobular carcinoma (ILC); both had three RE with margins persistently involved by ILC. The sizes of their ILCs on initial lumpectomy were 1.3 cm and 1.5 cm. The third patient had four RE with margins involved each time by invasive ductal carcinoma (IDC). Eleven patients achieved clear margins with BCT on their third RE. Initial lumpectomy specimens in these eleven patients demonstrated IDC (4 cases, all with extensive intraductal component) ranging from 0.18-4 cm, invasive carcinoma with mixed ductal and lobular features (IMC) (2 cases), 2.5 and 3 cm, and ductal carcinoma in-situ (DCIS) (5 cases) ranging from 0.8 cm. to 6 cm. For the patients with IDC, RE were performed for margins involved by DCIS (7 RE), IDC (2 RE), or both (3 RE). For patients with IMC, RE were performed for margins involved by IMC (3 RE), DCIS (1 RE), or both (2 RE). Follow-up is available in thirteen patients, none of whom have evidence of local recurrence (follow-up 10 to 73 months). One patient with a triple RE underwent reconstruction for cosmetic reasons.

Conclusions: A third RE was a rare occurrence. The average age of the study group is younger than that of breast cancer patients in general. 79% of patients who underwent a third RE achieved cosmetically acceptable BCT. ILC appears to increase the risk of mastectomy. While patients with IDC most often have DCIS at RE margins, patients with ILC and IMC most often have invasive carcinoma at RE margins. Additional follow-up is necessary to assess the risk of recurrence in these patients.

180 Beta-Catenin Is Activated in Residual Breast Carcinoma after Neo-Adjuvant Chemotherapy

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Background: Presence of residual carcinoma following neoadjuvant chemotherapy in patients with breast cancer is a powerful poor prognostic factor.Discovery of new molecular defects driving the aggressive behavior of residual carcinoma cells could result in targeted treatment and improved outcomes. Nuclear and/or cytoplasmic β-catenin accumulation indicating pathway activation is rare in primary breast carcinomas. We hypothesized that residual carcinoma following neoadjuvant chemotherapy have alterations in the Wnt/β-catenin pathway leading to poor differentiation and metastatic potential.

Design: Fifty-one post-neoadjuvant (post-NA) excisions, and matched 25 pre-treatment (pre-NA) core biopsies were studied. A tissue microarray with triplicate samples of the post-NA carcinomas and pre-treatment core biopsies were immunostained with anti-β-catenin antibody (BD BioSciences, San Diego, CA). Normal β-catenin was defined as crisp membrane staining in >90% tumor cells, whereas β-catenin accumulation in the nucleus and/or cytoplasm in >5% of cancer cells was considered aberrant. Reduced membrane staining was also recorded. Clinico-pathological information including tumor size, hormone receptors, HER-2/neu and lymph node status was available.

Results: Aberrant nuclear and/or cytoplasmic β-catenin was significantly more common in post-NA residual carcinoma (20/51, 39% vs.3/25, 12%, chi-square test, $p<0.02$). Reduced membrane β-catenin expression was common in pre and post-NA samples (17/25, 68%; and 21/51, 41% respectively). The median size of post- NA residual carcinomas with aberrant β-catenin was 1.3 cm (range 0.2-5 cm) vs. 1.5 cm (0.2-4.6 cm) in post-NA carcinomas with normal β-catenin expression. Aberrant β-catenin was significantly more frequent after NA chemotherapy in triple negative invasive carcinomas (60%), when compared to ER positive (22%) and HER 2/neu over-expressing tumors (25%), $p=0.02$. Aberrant β-catenin was not associated with lymph node metastasis.

Conclusions: Residual carcinoma cells resistant to conventional neoadjuvant chemotherapy have frequent activation of the Wnt/β-catenin signaling pathway. This phenomenon is common in triple negative tumors. This novel finding may have profound clinical implications in the treatment of patients with residual carcinoma after neoadjuvant chemotherapy which may lead to improved survival.

181 Experience with Oncotype DX at a Single Institution: Correlation with Histologic Tumor Features

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Background: OncotypeDX is an RT-PCR based assay that evaluates expression status of 21 genes in women with Stage I or II node- negative ER positive breast cancer. A recurrence score calculated from gene expression results using statistical modeling is translated into a recurrence risk percentage (RRP) that stratifies patients into low, intermediate and high risk for distant recurrence at 10 years. Oncologists recommend chemotherapy for "high"(>20%) recurrence risk while chemotherapy is optional for "intermediate"(>11%) recurrence risk.

Design: Invasive breast carcinomas (n=99) diagnosed at our institution with known OncotypeDX results were retrieved. Two pathologists (P1 and P2) independently assessed following histologic parameters: histologic type, Nottingham Grade, lymphocytic infiltration. Findings were correlated OncotypeDX RRP.

Results: Fifty three carcinomas were ductal, 12 lobular, 13 combined ductal/lobular, 9 papillary/micropapillary, 7 mucinous and 5 miscellaneous (3 tubular,1 metaplastic and 1 medullary). P1 recorded 58 Nottingham Grade 1, 28 Grade 2 and 11 Grade 3 cases while P2 recorded 54 Grade 1, 35 Grade 2 and 10 Grade 3 carcinomas (P1 and P2 agreed in 82/99 cases, kappa statistic 0.68, no disagreements in more than 1 grade). Most disagreements (16/17) were between Grade 1 and Grade 2. The Oncotype DX RRP ranged from 4%-34% (mean 12%) with 71% cases between 6-15%. There was significant correlation between Nottingham Grade and RRP for P1 ($p=0.006$) and P2 ($p=<0.001$). Nottingham Grade 1 correlated with low risk in 60% cases, Grade 2 correlated with intermediate risk in 48% cases and none of the Grade 3 cases correlated with low risk (P1 and P2). Prominent lymphocytic infiltrates were noted in 10% cases and correlated with increased OncotypeDX RRP ($p=0.0009$).

Conclusions: OncotypeDx appears to be a highly grade correlated parameter that would potentially add therapeutically useful data in 40% of Grade 1 and 60 % of Grade 3 cases (by assigning cases to "intermediate" RRP) and 52% of Grade 2 cases (by assigning cases to "low" or "high" RRP).

182 Secretory Breast Carcinoma: A Low Grade Basal-Like Carcinoma Associated with Translocation

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Background: Secretory breast carcinomas (SBC) (<1% of breast tumors) are associated with a characteristic morphology and a favorable prognosis. This entity is the only epithelial tumor of the breast with a balanced translocation, t(12;15), that creates a ETV6-NTRK3 gene fusion which encodes a chimeric tyrosine kinase also encountered in mesoblastic nephroma and infantile fibrosarcoma. The aim of this study was to determine the phenotypic class (ie luminal A/B, ERBB2, basal-like) of SBC.

Design: We evaluated 6 SBCs retrieved from Institut Curie files between 2000 to 2007 by morphological, immunohistochemical (IHC) methods and interphase FISH. IHC staining was performed for estrogen (ER) and progesterone (PR) receptors, HER2/neu, S100, SMA, p63, CK5/6, CK8/18, CK14, CD117, E-cadherin and EGFR. We used ETV6 split-apart probes for the detection of this translocation by fluorescence in situ hybridization (FISH) in paraffin-embedded, formalin-fixed tissue sections.

Results: The median age at presentation was 47 years (25-60 yo). Tumor size ranged from 5 to 40 mm (mean: 17 mm). The six cases presented SBC histological patterns including, solid, microcystic, and ductal patterns. The tumoral cells were polygonal with granular eosinophilic cytoplasm. Atypia was moderate to marked. Mitotic activity was nul or low (0-8 /10 HPF). A typical finding was the presence of intracellular and extracellular secretions. All tumors presented an in situ component of intermediate to high grade. Vascular and and perineural infiltration were absent. Four tumors were EE histologic grade II and two grade I. Four of the six cases of SBC showed ETV6 split apart signals in epithelial cells both in the invasive and *in situ* component. All secretory carcinomas were negative for ER, PR, HER2 (triple negative), expressed E-cadherin and focally CK8/18. 83% of SBC expressed focally CK5/6 (in more than 5% of the cells), SMA, diffusely S100 and vimentin. 66% of SBC expressed CD117 but none expressed p63. In situ and infiltrative component presented the same immunoprofiles.

Conclusions: SBCs are low-grade triple negative carcinomas that belong to the basal-like spectrum and harbor a translocation. This basal-like tumor is frequently associated with an *in situ* component. ETV6 gene alterations were present in both *in situ* and invasive component highlighting their genetic relationship. These results support the hypothesis that SBCs have immunohistochemical and genetic features that distinguish them from other basal-like tumors of the breast.

183 Failure of Intraoperative Margin Assessment in Breast Conserving Therapy of Invasive Breast Carcinoma Seems To Be Influenced by Associated DCIS

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Background: Intraoperative assessment of surgical margins (IAM) by frozen section (FS) is widely used but not generally accepted for breast conserving therapy (BCT) of invasive breast carcinoma (IBC). FS allows immediate re-excision but may also have disadvantages. The aim of this study is to determine the accuracy of margin analysis by FS and to find causes for failure of FS.

Design: 282 consecutive breast specimens from a single institution submitted for FS over a 2 year period were retrieved from our Pathology database. 168 specimens from 163 patients finally diagnosed as IBC were analyzed retrospectively. Median patients' age was 60 years (33-89 years). 34 patients (37 specimens) had neoadjuvant chemotherapy. IAM included gross examination of the sliced specimen and frozen section of 1-2 tissue blocks with the closest distance to the margins. For permanent sections (PS) multiple tissue blocks were taken. The minimal distance between tumour and margins in FS and PS was obtained from the original pathology report and by slide review. The margin status was categorized as involved (R-1), close (<1mm), free (≥1mm) and equivocal (R-x).

Results: The IBC was detected in 89.9% by FS. In 4.7% DCIS was diagnosed and in 5.4% no tumor was found by FS. Intraoperatively, the margins were involved in 12.5% and close in 14.3%, respectively. Immediate re-excision led to free margins in all but 4% of the cases. Intraoperative margin analysis failed in 18% as determined by PS (free margins in FS, involved or close margins in PS). The failure was caused by associated DCIS at or close to the margins in 60% and by IBC in 39%. IBC with a DCIS component showed more often failure (41.1%) compared to IBC without DCIS (8.7%) ($p<0.001$; chi2 test). Failure of intraoperative tumor detection was also associated with failure of IAM ($p=0.0001$) and was frequently caused by IBC ≤ 5mm and preoperative

chemotherapy. Lobular histology and preoperative chemotherapy were not associated with failure of IAM showing a trend for the latter. Subsequent re-excision or mastectomy showed residual DCIS in 35% and IBC in 27%.

Conclusions: Intraoperative FS of IBC margins may prevent additional surgery in 21% but may fail in 18% due to associated DCIS and failure of tumor detection. Preoperative management for IBC with associated DCIS needs to be improved to prevent additional operations. A close margin seems to be insufficient for BCT of IBC.

184 Mammary Paget's Disease Is Strongly Associated with PR Negative, HER2 Positive, and High-Grade Non-Basal Subtypes Ductal Carcinoma of the Breast

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Background: Mammary Paget's disease is a rare manifestation of breast carcinoma involving the nipple. Our objective is to identify molecular markers and molecular classifications that may predict which patients are at risk of developing Paget's disease.

Design: Immunohistochemical analysis was performed on representative sections of 27 cases of mammary Paget's disease and 87 high-grade carcinomas without Paget's disease (43 DCIS and 44 IDC) to antibodies against ER, PR, AR, HER2, EGFR, CK5/6, CK14, CK17, CK8 and CK18. The receptor expression rates and subtype distributions of 3 molecular classifications were compared between these two groups. All 3 molecular classifications divide tumors into basal and non-basal subtypes with basal subtypes defined as follows: CK5/6, CK14 and/or CK17 positive for Cytokeratin (CK) classification; ER, PR and HER2 negative for Triple Negative (TN) classification; and CK5/6, CK14, CK17, and/or EGFR positive and ER, PR, HER2 negative for CK/TN classification.

Results: The 27 cases of Mammary Paget's disease had a mean age of 58, with 14 associated with DCIS, 11 with IDC, and 2 with no known tumor. Most of the associated tumors were high-grade and ranging 1.1 cm. to 7.5 cm in size. The comparison between carcinomas with or without Paget's disease is shown in Table 1.

Comparison between breast carcinomas with or without mammary Paget's disease

	Carcinomas without Paget's Disease	Carcinomas with Paget's Disease	p-value
Receptor expression (%)			
ER +	21(26%)	4 (15%)	0.4268
PR +	21(26%)	0 (0%)	0.0032
AR +	64 (73%)	19 (70%)	0.8059
HER2 +	39 (45%)	24 (89%)	<0.0001
EGFR +	9 (10%)	1 (4%)	0.4475
Molecular Classifications (%)			
Cytokeratin (CK)			0.0020
Non-basal	58 (67%)	26 (96%)	
Basal	29 (33%)	1 (4%)	
Triple Negative (TN)			0.0748
Non-basal	61 (70%)	24 (89%)	
Basal	26 (30%)	3 (11%)	
CK/TN			0.0111
Non-basal	70 (80%)	27 (100%)	
Basal	17 (20%)	0 (0%)	

Conclusions: 1. Mammary Paget's disease is strongly associated PR negative, HER2 positive, and high-grade non-basal subtypes ductal carcinomas of the breast by CK and CK/TN classifications. 2. Expression rates of ER, AR, EGFR, and TN classification may not be useful in predicting Paget's disease.

185 Breast Cancer Tumorigenic Cells Are Resistant to Chemotherapy

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Background: Tumorigenic breast cancer cells characterized by CD44 expression and low or undetectable CD24 levels (CD44+/CD24-/low) may be resistant to chemotherapy and therefore responsible for cancer relapse.

Design: Paired breast cancer core biopsies before and after neoadjuvant chemotherapy or lapatinib treatment were obtained and single cell suspensions were made. These cells were stained using antibodies against CD24, CD44, and lineage markers; and then analyzed by flow cytometry. Mammosphere (MS) formation in culture was compared before and after treatment. Global gene expression differences between cancer cells bearing CD44+/CD24-/low cells and all other sorted cells, as well as between cancer MS and the primary bulk invasive cancers were analyzed.

Results: CD44+/CD24-/low tumorigenic breast cancer cells were intrinsically chemoresistant. Chemotherapy led to increased CD44+/CD24-/low cells, increased self-renewal capacity on MS assays, and enhanced tumorigeneicity in immunocompromised SCID/Beige mice. Conversely, in patients with HER2 overexpressing tumors, the EGFR/HER2 tyrosine kinase inhibitor, lapatinib did not increase CD44+/CD24-/low cells. After combined lapatinib and conventional chemotherapy treatment, the majority of these patients achieved pathologic complete response, a validated surrogate marker for long-term survival. From our microarray analysis of the largest data set to date, the gene transcription pathways that underly chemoresistant, MS-forming CD44+/CD24-/low cells involve genes belonging to stem cell self-renewal (polycomb group – PHC3, Notch pathway – mastermind-like 2, and NOTCH2N), FOXP1, epidermal growth factor/PI3K signaling (Sos, ITPR1, ITPR2, CK2, PI3K, Gab1/2, PTEN, PP2A), and early development pathways (JARID2, JMJD2C, and MBNL1).

Conclusions: This study provides the first clinical evidence that conventional chemotherapy fails to eradicate CD44+/CD24-/low cells which are intrinsically chemotherapy resistant, and suggests that specific signaling inhibitors of the pathways identified may provide a therapeutic strategy for eliminating these tumorigenic cells.

186 Expression and Function of Androgen Receptor Co-Activator P44/MEP50 in Invasive Ductal Carcinoma of Breast

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Background: The role of androgen and androgen receptor in the tumorigenesis and progression of invasive ductal carcinoma has long been recognized. P44 is a newly identified androgen receptor coactivator that enhances androgen receptor mediated transcriptional activity in a ligand dependent manner. This study characterizes the expression pattern of p44 in invasive ductal carcinoma. The correlation of the p44 expression pattern with other clinicopathological factors is also investigated.

Design: Immunohistochemical study with polyclonal antibody against p44 was used to characterize the expression pattern of p44 in 52 samples of invasive ductal carcinoma and 20 samples of control tissue on a tissue microarray (TMA). Each sample is represented by three 0.6 mm cores on the TMA. The levels of nuclear and cytoplasmic p44 expression were scored semi-quantitatively; 0 as negative, 1 as weak, 2 as moderate and 3 as strong expression.

Results: The expression of p44 in nucleus is significantly higher in breast cancer compared to benign breast glands. In particular, nuclear p44 is associated with the expression of androgen receptor ($p = 0.005$) and progesterone receptor ($p = 0.04$). The level of p44 nuclear expression is not correlated with age at diagnosis, tumor size, lymph node positivity, the status of estrogen receptor and HER2/neu. No correlation between the level of p44 cytoplasm expression and the above mentioned clinicopathological factors was observed.

Conclusions: The study demonstrated strong up-regulation of p44 nuclear expression in the subgroup of the invasive ductal carcinoma cases that express androgen receptor. The findings suggest that p44 plays a role in mediating the effects of hormone during tumorigenesis and progression of the invasive ductal carcinoma of the breast.

187 Percentage of High Risk Breast Lesions Upgraded after Core Biopsy: A Systematic Review and Meta-Analysis

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Background: Current guidelines of the National Comprehensive Cancer Network (2006) recommend excision of breast lesions that on core biopsy are diagnosed as "atypical hyperplasia, lobular carcinoma in situ (LCIS), papillary lesion, or other histologies of concern to the pathologist". These guidelines are not specific and do not mention the risk of carcinoma (ductal carcinoma in situ [DCIS] or invasive carcinoma) associated with each of these diagnosis. A meta-analysis was performed to estimate the percentage of cases upgraded to DCIS or invasive carcinoma after immediate surgical excision of cases diagnosed on core biopsy as atypical lobular hyperplasia (ALH), LCIS, papillary lesion, radial scar, or flat epithelial atypia (FEA).

Design: Relevant studies were identified using Pub med database. Studies included were those that provided sufficient data to calculate the percentage of cases upgraded in each category, and only those in which the association of more than one high grade lesion on the core biopsy was excluded. Cases with the diagnosis of pleomorphic LCIS or LCIS with necrosis were excluded.

Results: Fifty six articles were found and met the above criteria. The number of studies and patients included in the meta-analysis were: 21 and 245 for LCIS, 18 and 184 for ALH, 16 and 330 for papillary lesions, 7 and 662 for radial scars, and 6 and 182 for FEA. The papillary lesions and radial scars were subdivided into 2 categories: with and without atypia on the core biopsy. The percentage of cases upgraded to DCIS or invasive carcinoma in each category was 15% of LCIS, 12% of ALH, 8% of papillary lesions without atypia, 44% of papillary lesions with atypia, 4.8% of radial scars without atypia, 25% of radial scars with atypia, and 19% of FEA. Sixty percent of upgraded cases were DCIS, and forty percent were invasive carcinomas.

Conclusions: This meta-analysis confirms the need for surgical excision of cases with LCIS, ALH, FEA, radial scars with atypia, and papillary lesions with atypia. The need to excise radial scars and papillary lesions without atypia is controversial.

188 Is Measurement of Ki-67 in Axillary Lymph Node Metastasis of Breast Cancer Useful?

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Background: Several studies have proven the prognostic significance of cell proliferation in breast cancer and its positive relationship with tumor grade, size, mitotic activity, hormonal and Her-2 status and tumor pogression. The Ki-67 antigen is expressed in cycling cells, providing an accurate measure of the growth fraction of a tumor. The objective of this study was to investigate the prognostic significance of Ki-67 expression in axillary lymph node metastases as compared to the matched primary tumor.

Design: 103 breast carcinomas, consisting of 17 SBR grade I, 32 grade II, and 54 grade III, were evaluated using the ACIS system for Ki-67 expression in primary tumor and lymph nodes. Histologic types included 86% ductal, 5% lobular, 7% mixed ductal and lobular and 2% other types. Ki-67 percent expression was compared between primary tumor and lymph nodes, and correlated with age, grade, estrogen receptor (ER), progesterone receptor (PR), p53, epidermal growth factor receptor (EGFR), Bcl-2, Her-2 status, and patient overall survival.

Results: Mean Ki-67 in primary tumors was 28.7% and 26.3% in metastatic tumors. Overall Ki-67 expression in primary and metastatic lesions correlated positively with higher tumor grade, EGFR and Her2 status, and negatively with ER, PR, p53, Bcl-2 status and overall survival. When overall survival was calculated, there was no difference ($p=0.65$, log-rank test) between primary tumors with < or >10% Ki-67 expression. In contrast, there was significantly better overall survival when lymph nodes Ki-67 was <10% than when Ki67 was >10% ($p=0.040$). For 34 primary tumors with Ki-67 of <10%, the majority of their corresponding metastatic lesions also had a similar low

Ki-67 and a similar favorable outcome. A small subgroup of 6 was noted to have nodal Ki-67 >10% and worse survival (p=0.047). Similarly, when the 69 primary tumors with Ki-67 >10% were analyzed, it was noted that most of their metastatic lesions had similar high Ki-67 value. A group of 12 patients had <10% Ki-67 in lymph nodes and had a slightly better overall survival (p=0.092).
Conclusions: Our results showed that measurement of Ki-67 in lymph nodes is not only valuable but might be superior to evaluation of Ki-67 expression in primary tumor for predicting overall survival of patients with metastatic breast cancer. Identification of subgroups of patients where Ki-67 expression in lymph nodes differs from expression in primary tumor may assist in selection of therapeutic options.

189 BRCA1-Associated Ductal Carcinoma In Situ of the Breast Frequently Shows a Basal-Like Phenotype

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Background: BRCA1-associated invasive carcinomas have been shown to have common morphologic and immunophenotypic features including high-grade histology, hormone receptor (HR)-negative status, and a basal-like phenotype. Intrinsic subtypes of breast cancer including luminal A, luminal B, basal-like and HER2 overexpressing have been identified in both invasive and in situ carcinomas. The exact precursor lesions of HR-negative breast cancers remain unclear. The aim of this study was to immunohistochemically evaluate BRCA1-associated precursor lesions including ductal carcinoma in situ (DCIS) and atypical ductal hyperplasia (ADH) and compare their immunophenotype with the corresponding associated invasive carcinoma.
Design: Histologic sections from all biopsy/mastectomy specimens from patients in our institution with a confirmed germline BRCA1 mutation were reviewed. A total of 20 mixed DCIS and invasive carcinomas and 5 mixed ADH and invasive carcinomas were identified. Histologic features of the precursor lesions were noted including nuclear grade, necrosis, and lymphocytic response. Immunohistochemistry for ER, PR, HER2, EGFR, cytokeratin 5/6, cytokeratin 8/18 and vimentin was performed on the precursor lesions and corresponding invasive carcinomas. The basal-like subtype was defined as those cases showing an ER-, PR-, HER2-, cytokeratin 5/6+ or EGFR+ staining pattern.
Results: The histologic features of BRCA1-associated DCIS were similar to those observed in the corresponding invasive carcinoma. The DCIS cases showed grade 3 nuclei (19/20), necrosis (14/20), and lymphocytic host response (13/20). In contrast, all 5 ADH cases showed low-grade nuclei, absent necrosis, and absent lymphocytic response. In all 20 DCIS cases the immunophenotype of the DCIS component matched that of the invasive component. Nineteen of the 20 DCIS cases showed a triple negative phenotype (ER-, PR-, HER2-). One case was ER+, PR-, and HER2-. Sixteen (80%) of DCIS cases showed a basal-like phenotype. Seventeen (85%) of DCIS cases were positive for vimentin and 20 (100%) of cases were positive for cytokeratin 8/18. In contrast, all 5 of the ADH cases were ER+, PR+, cytokeratin 5/6-, EGFR-, and vimentin-.
Conclusions: BRCA1-associated DCIS, like its corresponding invasive component, frequently shows a basal-like subtype, high-grade histology, HR-negative status, and evidence of epithelial to mesenchymal transition. ADH cases in BRCA1 patients show preservation of hormone receptor status and negativity for basal-like markers.

190 KAI-1 Expression in Proliferative, Non-Neoplastic Lesions of the Breast

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Background: KAI-1 is a metastasis suppressor gene whose downregulation has, most notably, been shown to be associated with tumor progression in human prostate cancer. Decreased or absent expression has also been documented in many other tumors including esophageal, gastrointestinal, pancreatic, hepatocellular, ovarian, cervical, endometrial and bladder carcinomas. In addition, initial studies have suggested that KAI-1 expression may play a role in the metastatic potential and recurrence of breast cancer. Given the unique complexity of breast tissue with its many forms of neoplastic as well as non-neoplastic proliferations, the purpose of this study was to investigate KAI-1 expression in proliferative, non-neoplastic lesions of the breast.
Design: Paraffin-embedded sections from excisional breast specimens were retrieved from our institution's archival tissue. Nineteen sections showing typical fibrocystic changes and 17 sections showing columnar cell change were selected for study. Thirty-two sections of tissue showing histologically normal breast ducts/lobules were also included for comparison. All sections were subjected to immunohistochemical study using monoclonal anti-KAI1 antibody. Intensity and extent of immunoreactivity were recorded for each case using a scale of 0-3+ as follows: 0 (negative) = no staining; 1+ = staining of weak intensity and/or <50% epithelial staining; 2+ = moderate intensity with >50% epithelial staining; and 3+ = strong/diffuse epithelial staining.
Results: 78% of normal breast duct/lobule epithelium showed at least moderate immunoreactivity for KAI-1 expression and greater than one half (53%) showed strong/diffuse staining (3+). None of the 32 sections of normal breast tissue received a score of 0 (no staining). In contrast, 59% and 68% of columnar cell change and fibrocystic change, respectively, showed negative or only weak staining (0 or 1+). In addition, greater than half (53%) of the fibrocystic changes and 30% of columnar cell changes showed no staining (score of 0).
Conclusions: In addition to loss of KAI-1 expression in breast carcinomas, KAI-1 is also down-regulated in certain proliferative, but non-neoplastic breast epithelium. Given the myriad proliferative lesions of the breast -- including those currently considered non-neoplastic and potentially pre-neoplastic (i.e. ADH, FEA) -- evaluation of KAI-1 expression in all forms of proliferative breast epithelium may be useful in thoroughly understanding this metastasis suppressor gene's role in breast cancer and its progression.

191 Down-Regulation of KAI-1 Expression in Low Grade Ductal Breast Carcinomas with and without Lymph Node Metastases

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Background: KAI-1 is part of the transmembrane-4 superfamily whose members are involved in the regulation of cell proliferation, differentiation, and motility. Decreased expression of KAI-1 has been implicated in the progression/metastatic potential of many human carcinomas and has been demonstrated to be down-regulated in highly metastatic breast cancer cell lines and in specimens from highly agressive and recurrent breast carcinoma specimens. Very low grade breast tumors, such as purely tubular or cribriform carcinomas, are uncommon and based on morphologic parameters alone, are expected to pose a low risk for disease progression and metastasis. However, rarely these tumors do present with concurrent nodal metastases. The purpose of this study was to investigate the possible role of KAI-1 expression in very low grade invasive ductal carcinomas (IDC) of the breast, both with and without lymph node metastases.
Design: Low grade IDC was defined as tubular carcinoma, low grade IDC with tubular features, or cribriform carcinoma. Only tumors with an overall Modified Bloom Richardson grade of 1 were included. A search of our institution's database over a 10 year period identified 34 excisional breast specimens meeting criteria and having archived residual tissue available for study. Only 7 of these cases showed metastatic nodal disease in a total of 11 lymph nodes. Immunohistochemical studies using monoclonal anti-KAI1 antibody were performed on sections of each tumor and on each positive lymph node. Immunopositivity was evaluated based on intensity and extent of staining and scored on a 0 (negative) to 3+ (strong/diffuse staining) scale.
Results: In 32 of the 34 breast carcinoma sections histologically normal breast ducts/lobules were present for comparison. In 78% of these cases the normal breast tissue showed moderate (2+) or strong (3+) staining. Conversely, only 26% of the adjacent invasive tumors showed 2+ or 3+ staining and 74% of the tumors showed no (0) or only weak (1+) staining. In the 7 cases showing concurrent nodal disease 5 (71%) of the corresponding primary breast tumors showed negative (n=4) or weak/1+ (n=1) staining. All 11 lymph node metastases (100%) were negative.
Conclusions: Similar to findings reported in aggressive breast tumors, even very low grade IDCs of the breast show a significant decrease in KAI-1 expression. In addition, in cases with concurrent nodal disease, the metastatic tumor displays highly down-regulated or absent KAI-1 expression.

192 EGFR as a Prognostic Marker in Women with Hormone Receptor and HER2/neu Negative Breast Cancers

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Background: Women with estrogen receptor, progesterone receptor, and HER2/neu negative (triple negative, TN) breast cancers have a poor prognosis and fewer treatment options compared with patients whose cancers express these markers. A reported 54-72% of TN breast cancers express epidermal growth factor receptor (EGFR). We have generated data from a cohort of TN breast cancer patients suggesting that women with EGFR-negative tumors have a poorer prognosis than patients whose tumors are EGFR-positive. In this study, we will confirm these results in a larger cohort of patients.
Preliminary Data: EGFR immunostaining was performed on pre-therapy biopsy tissue from 30 TN, stage II-III breast cancer patients with known clinical outcomes. Fourteen were EGFR-negative and 6 (43%) of these tumors recurred. Of the 16 EGFR-positive tumors, only 1 (6%) recurred. Kaplan-Meier analysis showed that recurrence was more common and time to recurrence was shorter in patients with TN/EGFR-negative tumors, compared to patients with TN/EGFR-positive tumors (p= 0.028).
Design: We have identified 428 additional patients diagnosed with stage I-III TN breast cancers between 2000-2007 and treated at Siteman Cancer Center. Tissue microarrays are now being constructed by punching representative tissue from paraffin blocks of tumor and transferring to a recipient block. These will be immunostained for EGFR and expression will be evaluated by two pathologists. Results will be correlated with clinical data, including stage, disease-free survival, and overall survival.
Results: Clinical data is available on 414 patients. Of these, surgical specimens were available on 313 for tissue microarray construction. Sixty-nine of the 313 patients experienced recurrences. Based on our preliminary data, this additional sample set will provide 82% power at a 0.05 significance level to detect a difference between recurrence rates of 44% in EGFR-negative versus 25% in EGFR-positive patients, corresponding to a hazard ratio of 1.7.
Conclusions: Our preliminary data suggests that EGFR expression by immunohistochemistry in TN tumors is associated with a good prognosis. We expect these results to be validated in a larger cohort of patients. EGFR immunohistochemistry can then be used to stratify patients with TN tumors and identify candidates for targeted therapy.

193 Stat3, Stat3-pY^{705} and P-c-Jun Expression in Bening Breast Tissue, *In Situ* and Invasive Ductal Carcinoma of the Breast

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Background: Stats (Signal transducers and activators of transcription) are transcription factors involved in cell proliferation and differentiation. Data from cell lines, animal models, and human tissue suggest that Stat3 signaling is involved in the pathogenesis of breast cancer.
Design: We analyzed expression of Stat3, Stat3-pY^{705} and Stat transcriptional co-activator p-c-Jun in 40 cases of invasive ductal carcinoma (IDC), 30 cases of ductal carcinoma *in situ* (DCIS) and 10 cases of benign breast by immunohistochemistry using tissue microarrays. Cases were considered positive if more than 5 % of cells showed strong staining. Two authors independently read the slides and all the discrepancies

were resolved by a third pathologist. We used Chi-Square to analyze if expression of Stat3, Stat3-pY^{705} and p-c-Jun correlated with each other and with tumor size, grade, lymph node status, estrogen receptor (ER), progesterone receptor (PR) and Her2Neu expression.

Results: Stat3 staining was both nuclear and cytoplasmic while Stat3-pY^{705} and p-c-Jun stains were nuclear. All IDC, DCIS and benign breast epithelium were positive for Stat3. There was no significant difference in the intensity of staining among the groups (p=0.25). Fifty-two % of IDC (21/40), 36% of DCIS (11/30) and 90% of benign breast (9/10) were positive for Stat3-pY^{705}. Forty-five % of IDC (18/40), 63% of DCIS (19/30) 100% of bening breasat (10/10) were positive for p-c-Jun. Statistically significant number of IDC cases co-expressed Stat3-pY^{705} and p-c-Jun (p=0.041). Expression of Stat3-pY^{705} correlated with low and intermediate histologic grade in IDC using the Bloom-Richardson scale for grading breast cancer (p=0.0065). P-c-Jun expression did not correlate with histologic grade. Neither Stat3-pY^{705} nor p-c-Jun expression correlated with tumor size, lymph node status, ER, PR or Her2Neu receptor expression.

Conclusions: Expression and activation of Stat3 has been associated with both proliferation and involution of breast epithelium. Our data indicate that activation of Stat3 correlates with histologically better-differentiated tumors. They also suggest that Stat3 and c-Jun may operate along the same signaling pathway or mutually dependent signaling pathways in IDC of the human breast.

194 FOXA1: A Prognostic and Luminal Subtype A Marker in Breast Cancer

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Background: FOXA1 is a forkhead family transcription factor expressed in breast cancer cells. It is essential for optimum expression of ~50% of ER-related genes. We have previously reported that FOXA1 expression correlates with luminal subtype breast cancer and is a prognostic factor in these low-risk breast cancers. In this study, we explore FOXA1 relationship with proliferation and basal breast cancer markers in an entirely independent set of breast cancer patients who had received similar treatment.

Design: A tissue microarray comprising tumours from 245 similarly treated patients with 67 months of median follow-up was analyzed for FOXA1 expression by immunohistochemistry. Interpretable FOXA1 expression, obtained in 184 patients, was analyzed along with other variables like tumour grade, size, nodal status, ER, PR, HER2/*neu*, proliferation and basal markers.

Results: FOXA1 expression (score >3) was seen in 139 of 184 breast cancers. It correlated positively with ERa (p<0.0001), PR (p<0.0001), and luminal subtype (p<0.0001); negatively with basal subtype (p<0.0001), proliferation markers and high histological grade (p=0.0327). Univariate analysis showed nodal status, tumour grade, ER, PR, FOXA1, basal markers and p53 as significant predictors of overall survival (OS). Multivariate analysis showed only nodal status (p=0.0006) and ER (p=0.0017) to be the significant predictors of OS whereas FOXA1, tumour size, grade, and HER-2 were not significant. In luminal subtype patient subgroup, FOXA1 expression was associated with better survival (p=0.0284).

Conclusions: Based on this study in patients treated using single standard protocol, FOXA1 expression is a good prognostic factor. It correlates with luminal subtype breast cancer, and could possibly serve as a clinical marker for luminal subtype-A. Prognostic ability of FOXA1 in these low-risk breast cancers may prove to be useful in clinical treatment decisions.

195 Comparison of Estrogen Receptor and Progesterone Receptor Assay Results from Pathology Reports with Results from Centralized Testing: Implications for Population-Based Studies

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Background: Large, population-based studies of women with breast cancer commonly utilize information culled from pathology reports rather than central pathology review for practical and logistical reasons. The reliability of this information, particularly with regard to tumor biomarker results, is of concern.

Design: To address this, we compared estrogen receptor (ER) and progesterone receptor (PR) assay results determined from review of pathology reports with ER and PR results determined on the same specimens using immunohistochemical assays performed in a central laboratory. Tissue microarrays (TMAs) were constructed from paraffin blocks of 3,093 breast cancers that developed in women enrolled in the Nurses' Health Study. ER and PR immunostains were each performed on all TMA sections in single runs. Of note, the original ER/PR assays had been performed over a 20-year period in more than 40 laboratories.

Results: Among 1,851 invasive breast cancers in which ER results from pathology reports and central testing were both available, the reported ER status and the ER status as determined from immunostains on the TMAs were in agreement in 1,651 cases (87.3%; kappa value 0.64, p<0.0001). Agreement for PR results was slightly lower (80.9% agreement; kappa value 0.59, p<0.0001). When the comparison was restricted to cases in which the ER/PR assays were originally performed by immunohistochemistry, the agreement rate increased to 92% for ER (kappa value 0.78, p<0.0001) but remained essentially unchanged for PR (agreement 80.2%, kappa value 0.55, p<0.0001).

Conclusions: We found a high level of agreement between ER assay results abstracted from pathology reports with those obtained by central laboratory testing. The level of agreement for PR results was somewhat lower than for ER. These observations suggest that utilizing ER assay results from pathology reports is a reasonable, albeit imperfect, alternative to central laboratory ER testing for population-based studies of patients with breast cancer.

196 Expression of Estrogen Receptor (ER)-β in Invasive Breast Cancers in Relation to Molecular Phenotype

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Background: Distinct subsets of invasive breast cancer have been identified by their patterns of gene expression, including luminal (A and B), HER2 and basal types. Using this approach, expression of ER-α and related genes has emerged as one of the major determinants of the molecular classification. However, patterns of expression of a second ER, ER-β, have not been previously evaluated in detail among these molecular categories.

Design: We constructed tissue microarrays (TMAs) from paraffin blocks of 3,093 breast cancers that developed in women enrolled in the Nurses' Health Study between 1976-1996. TMA sections were immunostained for ER-α, PR, HER2, CK5/6 and EGFR. Results of these stains were used to categorize each cancer as luminal A (ER+ and/or PR+ and HER2-); luminal B (ER+ and/or PR+ and HER2+); HER2 (ER- and PR- and HER2+); and basal-like (ER-, PR-, HER2- and EGFR or CK5/6+). TMA sections were also immunostained with a monoclonal antibody to ER-β (ER-β1, clone PPG5/10, Serotec). The relationship between expression of ER-β and molecular class was analyzed.

Results: Overall, 69% of cases were ER-β positive. Expression of ER-β was significantly associated with expression of ER-α (p<0.0001) and PR (p<0.0001), and was inversely related to expression of HER2 (p=0.008), CK5/6 (p=0.0008) and EGFR (p=0.002). Among 2,110 cancers with complete immunophenotypic data, 77% were luminal A, 6% luminal B, 6 % HER2 and 11% basal. ER-β expression was significantly related to molecular category (p<0.0001) and was more common in luminal A (72% of cases) and B (68% of cases) than in HER2 or basal-like types. However, despite their being defined by the absence of ER-α expression, 55% of HER2-type and 59% of basal-like cancers showed expression of ER-β.

Conclusions: ER-β expression is commonly seen in luminal A- and B-types of invasive breast cancer. However, expression of ER-β is also seen in a subset of HER2 and basal cancers, despite the absence of ER-α expression in these tumors. The potential role of ER-β in the development and progression of invasive breast cancers defined by lack of expression of ER-α merits further investigation.

197 Clinicopathologic Features of Breast Carcinomas in Women Taking Postmenopausal Hormone Replacement Therapy

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Background: Postmenopausal combined hormone replacement therapy (HRT) has been shown to be associated with a slightly increased risk of being diagnosed with breast carcinoma (BC). Some epidemiologic studies using registry data have suggested that HRT use may also be associated with certain histological tumor types and low tumor grade. However, these studies are limited due to their lack of pathologic review of the cases. We compared the clinicopathologic features of invasive BC diagnosed in women taking HRT to those of non-users and premenopausal women in a large cohort of well characterized tumors.

Design: We selected 340 invasive BC diagnosed in postmenopausal women for the study. Among these 181 patients never used HRT, 91 patients were current users at the time of diagnosis (34 estrogen only, 57 combined HRT, median duration of use: 4.2 years), and 68 patients were prior (stopping >3 months prior to diagnosis) users (20 estrogen only, 48 combined HRT, median duration of use 5.1 years). All H&E slides were reviewed and the diagnoses confirmed including histologic type and grade. The clinicpathologic features of BC in the various groups were compared to one another and to those of 176 tumors in premenopausal women.

Results: The results are summarized in Table 1.

Summary of clinicopathologic features

		Never users	Prior users	Current users	Premenopausal	p*	p**
Median age (ys)		62	63.5	61	44	>0.05	<0.0001
Median tumor size (cm)		1.7	1.7	1.7	2.0	>0.05	>0.05
Histologic type	Ductal	153	52	76	156	>0.05	>0.05
	Mixed	5	4	4	5		
	Lobular	23	12	11	15		
Grade	Low	39	15	21	23	>0.05	0.036
	Intermediate	90	35	45	78		
	High	52	18	25	75		
Nodal metastasis	Absent	100	41	50	86	>0.05	>0.05
	Present	70	23	38	79		
ER	Positive	150	53	69	125	>0.05	0.067
	Negative	31	15	22	51		
PR	Positive	124	43	53	110	>0.05	>0.05
	Negative	57	25	38	66		
HER2	Positive	19	7	14	44	>0.05	<0.0001
	Negative	162	61	77	132		

* postmenopausal only; ** including premenopausal cases

All current users stopped HRT after diagnosis of BC on core needle biopsy. We found no difference in the mitotic activity (number of mitoses per 10 hpf) of BC determined in core biopsies and corresponding subsequent excisional biopsies.

Conclusions: Our results on a cohort of pathologically well characterized BC do not support the hypothesis that BC diagnosed in women taking HRT are different form those of non-users.

198 Molecular Analysis of KIT and Epidermal Growth Factor Receptor in Adenoid Cystic Carcinoma of the Breast

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Background: Adenoid cystic carcinoma (ACC) of the breast is a rare tumor that expresses a basal immunophenotype, despite its favorable outcome. KIT and epidermal growth factor receptor (EGFR) are more commonly expressed in basal type breast cancer than in other subtypes. Although overexpression of KIT in ACC has been reported, *KIT* activating mutations remain uninvestigated. Neither protein expression, mutational analysis, nor gene amplification of EGFR in ACC has been studied. Mutations in *KIT* exons 9, 11, 13, and 17 are associated with response to tyrosine kinase inhibitor (TKI) imatinib and mutations in *EGFR* exons 18, 19, and 21 with response to TKI gefitinib. We sought to determine whether tumor cells of ACC have evidence for *KIT* and *EGFR* activating mutations and/or *EGFR* gene amplification.

Design: Eighteen cases of ACC of the breast with available paraffin-embedded tumor blocks were identified in our surgical pathology consultation files from 1998 to 2006. DNA was isolated from all cases resulting in 18 unique aliquots of DNA for mutational analysis. The analyzed *KIT* and *EGFR* exons were amplified by PCR and amplicons were sequenced on both strands and assessed for activating mutations. Tissue microarray (TMA) sections were analyzed by FISH for *EGFR* amplification. *EGFR* FISH was performed with the LSI EGFR/CEP7 Probe (Vysis). Thirty nuclei were scored per sample and the ratio of EGFR (red) signals to CEP 7 (green) signals was recorded. *EGFR* FISH positive samples were those demonstrating amplification (EGFR/CEP7 ratio > 2). In addition, TMA sections were analyzed by immunohistochemistry for EGFR. Positive EGFR staining (1+, 2+, or 3+ intensity) was defined as any staining of tumor cell membranes (≥1%) above background level whether it is complete or incomplete circumferential staining.

Results: No *KIT* or *EGFR* activating mutations were identified in any of the samples and none displayed *EGFR* amplification. However, all cases demonstrated EGFR protein expression, with 62% of cases showing 3+ or 2+ staining intensity.

Conclusions: Although protein expression of KIT and EGFR are present in ACC, *KIT* and *EGFR* activating mutations and/or *EGFR* gene amplification predictive of responsiveness to tyrosine kinase inhibitors are not present.

199 Lobular Involution: A Quantitative Trait Directly Linked to Breast Cancer

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Background: The degree of age-related involution of terminal duct lobular units in a women's breast tissue is associated with the development of breast cancer. In a previous study done by our group, it was shown that women who had "complete involution" (75-100% of lobules demonstrating involution) had a decreased risk of developing breast cancer later in life. Alternatively, women with "no involution" (0%) were at higher risk. A large group of women were defined as having "partial involution" (1-74%). Because the approach was qualitative and had a wide range of involution in the "partial" involution group, we decided to test a quantitative assessment of lobular involution as a means of risk prediction.

Design: We performed a nested case control study with the Mayo Benign Breast Disease Cohort (BBD). The BBD database is a collection of breast tissue on all women who had open breast biopsies at Mayo between 1967 and 1991 and who were found to have benign breast disease. Subsequent outcome data has been obtained for these women. In this study, 86 cases and 152 controls were selected for a total number of 238. Approximately two controls to each case were matched to age and time period of biopsy. Hematoxylin and Eosin stained slides were scanned into the computer and analyzed using Webslide™ browser software (Bacus labs product). The 10 largest normal lobules were analyzed for each case. Analysis included area of the lobule and number of acini per lobule. Mean number of acini and mean lobule area were compared for cases and controls, involution status (none, partial, or complete), histology (NP, PDWA, or AH), and family history (none, weak, or strong).

Results: Women who went on to develop breast cancer had a larger lobular area (59458 μ^2 vs 49221 μ^2; p=0.0452) and higher number of acini per lobule (21.62 vs. 16.11; p=0.0006) than women who did not develop cancer. Women with no involution had a larger lobule area (102013 μ^2) and number of acini per lobule (35.72) than women with partial (56945μ^2, 20.85 acini per lobule) or complete involution (27254μ^2, 8.72 acini per lobule)(p<0.0001) The difference between lobular area and number of acini per lobule was not significantly significant when evaluating for effect of histology or family history (p=0.153 and 0.4770) respectively.

Conclusions: Lobular involution can be quantified and may be useful as a risk predictor for women who have had benign breast biopsies.

200 Performance of Intraoperative Axillary Sentinel Lymph Node Touch Prep Evaluation: Effect of Surgical Submission of "Suspicious" Sentinel Lymph Nodes

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Background: Touch preparation evaluation (TPE) of axillary sentinel lymph nodes (SLN) is the preferred method of intraoperative SLN assessment for the presence of metastatic breast carcinoma. As the prevalence of positive SLN is relatively low, the percentage of all SLN patients who are spared the morbidity of an additional procedure (completion lymphadenectomy) is limited, with added risk for potential false positive interpretation. The aim of our study is to examine the outcome of surgical submission of selected "suspicious" SLN on the performance of intraoperative TPE (I-TPE).

Design: Prior to this study (pre-selective I-TPE), all axillary SLN were routinely examined by I-TPE at our institution. All surgeons performing SLN for breast cancer were requested to submit only "suspicious" SLN for I-TPE (selective I-TPE), based upon their clinical assessment. Selective I-TPE vs. final SLN pathology results over 18 months were retrospectively compared to the same time period pre-selective I-TPE. Final SLN positivity was defined as either micrometastatic or macrometastatic disease. Isolated tumor cells identified by IHC were considered negative. Statistical analysis was performed by Chi-square test.

Results: A total of 330 patients underwent SLN biopsy for breast cancer during the study period (pre-selective I-TPE=147; selective I-TPE=183), involving 15 surgeons. Mean patient age and range were similar (mean 57 years). Clinical SLN assessment reduced the percentage of patients with SLN submitted for I-TPE (32% vs. 100%; $p<0.01$) with an increase in the proportion of patients with positive SLN (37% vs. 21%; $p<0.001$). When performing pre-selective I-TPE, the positive predictive value (PPV) and specificity (as examined per patient) were 92% and 98%. With selective I-TPE, these increased to 100% each.

Conclusions: Surgical assessment for "suspicious" SLN led to a decrease in patient SLN cases submitted for intraoperative TPE (I-TPE) with an increase in the relative proportion of I-TPE patients with positive SLN. This latter effect, by increasing the prevalence of SLN positivity in the tested patient population, aided in reducing the potential of a false positive I-TPE.

201 BRCA1 and BRCA2-Associated Breast Cancers: Morphological and Immunohistochemical Differences

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Background: Germline mutations in BRCA1 and BRCA2 result in familial breast and ovarian cancers. Both genes function in the DNA repair pathway and the resultant breast cancers have been shown to share a number of common characteristics: early age of onset, frequent bilaterality and predominant ductal phenotype. However, at a molecular level they appear to be quite divergent. To further characterize these two tumor types we performed a comprehensive comparative review of BRCA1 and BRCA2 breast cancers using tissue microarrays (TMAs).

Design: TMAs were constructed from tumors from 58 BRCA1 carriers and 64 BRCA2 carriers selected from the Ontario Familial Breast Cancer Registry. Immunohistochemical analysis of ER, PgR, HER2, p53, CK5, CK8/18, MIB-1, CK14, p27, cyclin D1 and vimentin was performed on the TMAs from both carrier groups and scored using the Allred method. Morphological and biomarker parameters were compared between the two groups using a Chi-square test or Fisher's exact test.

Results: BRCA1 and BRCA2-carriers showed no significant difference in age at diagnosis or tumor type (invasive ductal, no special type in 92 % and 96 %, respectively). There was no difference in tumor size, margin type (infiltrative vs. pushing) or presence of lymphovascular invasion. However, BRCA1-associated breast tumors were more often of higher grade (p=0.005), had higher mitotic scores (p=0.0334) and were less likely to show lymph node metastases (p=0.048). Immunhistochemical profiling of both tumor groups showed significant differences: BRCA1-associated tumors were more likely to be negative for ER (p<0.0001), PR (p<0.0001), p27 (p<0.0001), CK8/18 (p<0.0001) and Cyclin D1 (p<0.0001) and were more likely to be positive for p53 (p=0.0001), CK5 (<0.0001) and CK14 (p=0.0085). The two tumor groups did not vary significantly with regard to HER2, Ki-67 or vimentin expression.

Conclusions: BRCA1 and BRCA2-associated tumors have many morphological features in common, although BRCA1-associated carcinomas are more likely to be lymph node negative than BRCA2-associated carcinomas. The distinct molecular signatures of both tumor groups (BRCA1-associated tumors showing a basal phenotype and BRCA2-associated tumors showing a luminal phenotype), may play a role in the patterns of spread of these tumors.

202 Atypical Ductal Hyperplasia in Directional Vacuum-Assisted Biopsy of Breast Microcalcifications: Considerations for Surgical Excision

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Background: The reported frequency of finding carcinoma on surgical excision following diagnosis of ADH on directional vacuum-assisted needle biopsy (DVAB) varies from 0 to 38%. In this study, we aim to 1) review DVABs targeting calcification with ADH to correlate with findings on additional surgery or clinical/radiologic follow-up and 2) identify histologic features that would predict which lesions should be excised.

Design: In 768 needle biopsies coded as 'atypical' in our pathology database (1998 to 2007), there were 232 DVABs for microcalcifications. 105 cases were available for review by two breast pathologists. Twenty cases were excluded due to absence of ADH, misclassification of ADH, or presence of DCIS. DVABs were evaluated for extent of ADH (# of terminal duct-lobular units [TDLU]) and histologic features. Cases were categorized as minimal (ADH restricted to ≤3 TDLUs) or borderline (ADH involving ≥4 TDLU or with significant micropapillary or cribriform pattern). Segmental or total mastectomy specimen (SX) and clinical/radiologic data were reviewed for presence of carcinoma.

Results: A total of 85 cases of ADH with either excision or clinical/radiologic follow-up were evaluated. Forty-one (17 borderline, 24 minimal) of 85 cases had SX. Only 10 (8 borderline, 2 minimal) of 41 showed carcinoma on SX (8 DCIS; 2 invasive cancer). Although 2 of the DCIS were evaluated as minimal on DVAB, in one case excision was recommended due to residual calcifications and in the second case Paget's disease of the nipple was present. Forty-seven percent (8/17) of the borderline cases showed carcinoma on SX. In contrast, only 8% (2/24) of minimal cases showed carcinoma on SX. Of the 44 that did not have SX (37 minimal, 7 borderline), 25 showed no new lesions on follow-up (1-108 mos; average 21), 8 are pending surgical consults, and 11 were lost to follow-up. Of the 7 borderline lesions, 1 refused excision, 3 had no radiologic residual lesion on follow-up, 1 was lost to follow-up, and 2 are pending surgical consult.

Conclusions: We conclude that the extent of ADH correlates with the presence or absence of higher risk lesions (DCIS, invasive carcinoma) upon subsequent excision provided that radiological assessment indicates that the calcifications are adequately sampled. Reporting the extent of involvement of lobules or ducts and their histologic features and correlation with clinical and radiologic evaluation may be of help in making a decision as to whether or not an excision is warranted.

203 Mutational Profile of *RAS* and *BRAF* Genes in Papillary Lesions of the Breast: Identification of a Common *HRAS* Mutation in Intracystic Papillary Carcinoma

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Background: *BRAF* and *RAS* family gene mutations are reportedly rare in human breast cancer, though they have been shown to be diagnostically and prognostically important in other human carcinomas, such as papillary thyroid cancer. However, these reports have not examined papillary lesions of the breast, an often diagnostically challenging group of lesions in which identification of mutations may be diagnostically useful. The aim of this study was therefore to screen benign, atypical, and malignant papillary lesions of the breast for mutations in *BRAF* and the *RAS* family genes.

Design: Cases selected from the pathology archives included benign papillomas, atypical papillomas, papillary ductal carcinoma in situ (DCIS), and intracystic papillary carcinoma. Representative paraffin-embedded tissue blocks were cut at 4-microns, targeted lesions were manually microdissected, and DNA was extracted. Detection of *NRAS* codon 61, *KRAS* codon 12/13, *HRAS* codon 61, and *BRAF* V600E mutations was performed using real-time polymerase chain reaction (PCR) amplification and post-PCR fluorescent melting curve analysis.

Results: Six benign papillomas, three papillary DCIS, one atypical papilloma, and three intracystic papillary carcinomas were analyzed. No *NRAS*, *KRAS*, *HRAS*, or *BRAF* mutations were identified in the benign papilloma, papillary DCIS, or atypical papilloma groups. Two (66.7%) intracystic papillary carcinomas demonstrated an identical point mutation (CAG→AAG) in codon 61 of the *HRAS* gene.

Conclusions: *BRAF* and *RAS* gene mutations are uncommon in papillomas and papillary DCIS of the breast. However, the identification of a previously undescribed activating *HRAS* point mutation in two of three intracystic papillary carcinomas of the breast suggests they bear a distinct pathogenetic mechanism. Additionally, analysis for *HRAS* gene mutations in equivocal lesions may be diagnostically useful, especially in small biopsy specimens.

204 Analysis of Allelic Imbalance in Phyllodes Tumors of the Breast

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Background: Currently, phyllodes tumors (PTs) are classified as benign, borderline or malignant based on histopathologic features. However, histologic classification does not always predict outcome. This study used genetic profiling across a series of tumor suppressor genes to analyze the frequency of allelic imbalance (AI) in these tumors.

Design: Benign, borderline, and malignant PTs were included and the initial pathologic diagnosis was confirmed for this IRB-approved study. Patient follow-up data were retrieved from the medical record. Tumor was microdissected, DNA was extracted, and fluorescence based polymerase chain reaction (PCR) targeting 16 short tandem repeats was performed. PCR products were detected using semi-quantitative capillary gel electrophoresis. The height of the allele peaks in tumor samples was compared to normal values obtained by averaging five random blood samples from the general population. Ratios of <0.60 or >1.90 was considered to indicate the presence of AI. Fractional allelic loss (FAL) was calculated as the number of loci with AI divided by the number of informative loci.

Results: 10 benign (including 1 recurrent), 6 borderline, and 13 malignant (including 7 metastases) PTs were selected to be included. Mean and median follow-up times were 10.8 years and 4.1 years, respectively. Mean FALs were higher in borderline (43%) and malignant (28%) tumors versus benign (10%) tumors. AI at D6S297 (6q27) and D16S3140 (16q21, *matrix metalloproteinase-2*/*MMP-2* locus) was observed in borderline and malignant tumors only, and AI at the *p53* locus (17p13) was seen in only 1 malignant tumor. One benign PT that locally recurred and 1 malignant PT that metastasized demonstrated additional losses at 17q21 and 10p15, respectively.

Conclusions: High frequency of allelic loss was found in malignant and borderline phyllodes tumors as compared to benign tumors. Unique allelic losses in borderline and malignant PTs suggest (1) the 6q27 locus may harbor a candidate tumor suppressor gene, and (2) *MMP-2* may play a role in stromal proliferation and/or tumor development. The additional losses seen in recurrent/metastatic tumors compared to their corresponding primaries also suggests cumulative AI plays a role in tumor progression.

205 Incidence of Medullary Carcinoma vs Other Histologic Types in Triple Negative and HER2+ Breast Carcinoma

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Background: Hormone receptor negative breast cancers are generally high grade poorly differentiated cancers that may be Her-2/neu negative (triple negative) or Her-2/neu positive (Her2+). Triple negative (TN) breast cancers are known to have more aggressive behavior. Medullary carcinoma (MC) is also a hormone receptor negative poorly differentiated carcinoma that is regarded as having good prognosis. At present it is not clear if MC is an entity with good prognosis or a TN cancer with poor prognosis.

Design: ER/PR negative breast cancers (2001-2005) were identified and divided into TN and HER2+ groups. All H&E slides were reviewed and the tumors were classified into medullary (MC), atypical medullary (AMC), and non-medullary (NMC) subtypes. Based on previous definitions, MC features included >75% syncytial growth pattern, circumscribed margin, mod-marked lymphoplasmacytic infiltrate, intermediate to high grade nuclei, and no DCIS. AMC features were >75% syncytial growth pattern, and no more than two of the following: infiltrative margins, DCIS, no-mild stromal infiltrate, low grade nuclei, and tubule formation. All the others were NMCs. Lymph node (LN) status and clinical outcome were collected for each case with a follow-up of 0.5 - 80 mo. (median-38 mo.) Adverse outcome (AO) was defined as recurrence+/- death. Statistical analysis of contingency tables was performed using Fischer's exact test; survival analysis utilized log rank tests.

Results: Of 196 cases, there were 11(6%) MC, 32(16%) AMC, and 153(78%) NMC. All MCs (100%) were TN, 3(27%) had nodal mets, and all (100%) were alive without recurrences. In the AMC, 27(84%) were TN, 10(31%) had nodal mets, 28(88%) were without recurrences, and 4(12%) had AO. In the NMCs, 118(77%) were TN, 53(35%) had nodal mets, 117(76%) were without recurrences, and 36(24%) had AO. LN status was associated with recurrence in both contingency and survival analysis ($p<.0001$). Histologic subtype was not significantly associated with any other variables.

Conclusions: Results show that: 1) Among hormone receptor negative breast carcinomas, Her2+ tumors are fewer and none showed medullary features. 2) Although few in number (16%), TN tumors with medullary features had the least nodal metastasis and appeared to have better outcome than AMC and NMC phenotypes. 3) Non-medullary triple negative tumors had the most nodal mets (37%) and (24%) had adverse outcomes. 4) Lymph node status, but not histologic subtypes, was significantly associated with recurrence.

206 HER-2/neu Splicing Variant Expression in Human Breast Cancer: Comparison of RT-PCR and Immunohistochemistry and Fluorescence In Situ Hybridization

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Background: HER-2/neu is both a prognostic marker and a therapeutic target in human breast cancer. Currently, the HER-2/neu status is estimated by fluorescence in situ hybridization (FISH) test and immunohistochemistry (IHC) test in routine practice. Although the HER-2/neu expression at the mRNA levels is estimated by quantitative real-time RT-PCR (qRT-PCR), it is reported that the case in which the result of FISH and IHC differs from qRT-PCR. The aim of this study is to evaluate the expression of HER-2/neu mRNA splicing variants in human breast cancers by RT-PCR and to compare it with results of FISH and IHC.

Design: Tumor was collected from 46 patients diagnosed with invasive breast cancer. After the surgery extraction, tissue was fixed in the 10% formaldehyde at 4°C. The fixed tissue was embedded in the paraffin, and stored at 4 °C. The paraffin-embedded sections were analyzed by IHC, FISH and RT-PCR. The HER-2/neu mRNA splicing variants data was acquired from EMBL database and NCBI database, and the expression pattern of splicing variant was analyzed by RT-PCR.

Results: 8 of 46 cases showed HER-2/new gene amplification by FISH, and showed HER-2/new protein over expression by IHC (scored 3+/1case, 2+/4cases, 1+/3cases). 11 of 46 cases showed no HER-2/new gene amplification by FISH, but showed HER-2/new protein over expression by IHC (scored 2+/4cases, 1+/7cases). 27 of 46 cases showed no HER-2/new gene amplification by FISH, and showed HER-2/new protein negative by IHC. Although, all of these 46 cases showed expression of multiple HER-2/neu mRNA splicing variants, they did not show characteristic expression pattern which correlates with the result of FISH and the result of IHC.

Conclusions: Thus we arrived at the conclusion that both HER-2/new gene amplification and HER-2/new receptor over expression was unrelated to expression patterns of HER-2/neu mRNA splicing variants. However, there was the high-frequent expression of the HER-2/neu mRNA splicing variants from the clinical samples, the primer design without other splicing variant are necessary for the accurate analysis of HER-2/neu mRNA by qRT-PCR.

207 Androgen Receptor Expression in Breast Carcinomas Arising in BRCA1 and BRCA2 Mutation Carriers

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Background: Androgen receptor (AR) expression has been reported in over 80% of sporadic invasive breast carcinomas. AR may have a role in the pathogenesis of hereditary breast cancer. Specifically, previous studies have shown that BRCA1 may modulate AR signalling. In this study our aim was to evaluate the frequency of AR in tumors from BRCA1 and 2 carriers and controls and to examine the association of AR with previously evaluated immunohistochemical markers.

Design: Sections from tissue microarrays constructed using cases from the Ontario Familial Breast Cancer Registry were stained with the AR antibody. Nuclear staining was assessed using the Allred method (≥ 5 = positive). Immunohistochemical staining for ER, PR, CK5, CK14, EGFR and HER2 had previously been performed. The results were analysed using Fisher's exact test.

Results: 23 invasive carcinomas from BRCA1 carriers, 25 from BRCA2 carriers and 100 controls were suitable for interpretation. Table 1 summarizes the frequency of AR in the 3 groups. Expression of AR was significantly different between BRCA1 and BRCA2 tumors ($p=0.0003$) and between BRCA1 and control tumors ($p < 0.0001$), but not between BRCA2 and control tumors. The results remained statistically significant even after adjusting for multiple testing.

Table 1. AR and Tumor Type

AR	BRCA1		BRCA2		Controls		P-value
	n	%	n	%	n	%	Chi-square test
Positive (5-8)	3	13.0	16	64.0	67	67.0	p<0.0001
Negative (0-4)	20	87.0	9	36.0	33	33.0	

Basal breast cancers were more likely to be AR negative than AR positive (87.8 % vs. 12.2%; P<0.0001). AR expression in the BRCA1-associated and control tumors was positively associated with ER and PR (P<0.05). AR negative tumors in the control group were more likely to be positive for EGFR, CK14, and vimentin (p<0.05) than AR positive tumors (p<0.05). In all tumor groups AR expression was negatively associated with CK5 expression. There was no association between AR and HER2 positivity.

Conclusions: Our study shows that the frequency of AR expression in BRCA1-associated tumors and sporadic basal type tumors is low. In contrast, BRCA2-associated tumors are more likely to express AR. The potential therapeutic implications of these findings require further study.

208 Concordance of the Her-2/neu Status in Paired Primary and Metastatic Breast Cancers: Should We Retest the Metastatic Tumors?

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Background: To date the hormonal (HR) and HER-2/neu status are prognostic and predictive factors essential for the management of breast cancer both in the adjuvant and metastatic setting. Discordance between the HER-2/neu status of the primary and subsequent metastasis varies from 0-14%, while for HR it varies for 15-37%. This might represent true discordance or is related to non-standardized methodology. The aim of this study was to compare the HER-2/neu status of paired primaries and metastasis in a large cohort of cases with concurrent assessment of the HR status. We excluded local and regional recurrences.

Design: Paraffin blocks from 90 patients with corresponding primary and metastatic tumors were retrieved from the surgical pathology archives. The metastases were from skin (34), lung (5), neck lymph nodes (12), bone (11), liver (8), brain (6), pleura (2), peritoneum (2) and 10 other different organ metastases. HER-2/neu status was assessed with immunohistochemistry (IHC) using standardized methodology. Equivocal cases were tested with FISH (PathVysion Kit) using the scoring criteria of the latest ASCO/CAP and Canadian guidelines. Tumors were scored as positive for HR if >1% of cells were positive.

Results: HER-2/neu was discordant in 3/90 (3.3%) of cases. 2/3 Her-2/neu cases had a positive primary and a negative skin metastasis. The third case was a positive skin metastasis with negative primary. ER was discordant in 17/90 (18.8%) of cases (p<0.01). 11/17 cases were primary positive and metastases negative, 6/17 were primary negative and metastases positive. PR was discordant in 26/90 (28.8%) of cases (p<0.01). 21/26 PR cases were primary positive and metastases negative and 5/26 primary negative and metastases positive.

Conclusions: The use of standardized methodology for HER-2/neu testing showed that the rate of HER-2/neu discordance between the primary and the metastasis is low (3.3%) suggesting that the HER-2/neu status is relatively stable and treatment decision could be based on the HER-2/neu status of the primary. However the high level of discordance for ER (18.8%) and PR (28.8%) requires further evaluation if hormonal treatment is considered in the management of metastatic disease.

209 Pathological Response to Neoadjuvant Chemotherapy in the Triple Negative Breast Cancers in Our Patient Population

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Background: Triple negative tumors (TNT) don't express the estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor type 2 (HER), depriving the oncologist of therapeutic target. They are especially frequent in young African-American women. These tumors have an unfavorable prognosis. The effect of neoadjuvant chemotherapy (NC) for breast cancer has been extensively studied. The aim of our study was to evaluate the histopathological features of TNT after NC.

Design: TNT breast cancers diagnosed in our hospital during 2003-2007 was searched. The diagnostic and excisional slides were reviewed for cellularity, macrophages, lymphocytes, presence of necrosis and cyto-histologic appearance of tumor after NC. The study included age, race, tumor size, node status.

Results: For 32 cases slides were available for review. Tumor size ranged from 1.8 to 12 cm with a median of 3.5 cm and a mean of 4.2 cm. Number of nodes involved by tumor ranged from 0 to 19 with a mean of 4.5. 16 patients were node negative. Age range was 36-73 years and 94% were African-American. TNT equally distributed in the pre and postmenopausal age. Characteristics of the 26 patients who received NC are presented in table 1.

Table 1. Tumor cellularity after neoadjuvant chemotherapy by age

Age	Complete response	Non-Responders	Cellularity < 30%	Cellularity 31-69%	Cellularity >70%	Total
Less than 50	3	1	5	1	3	13
Greater than 50	2	1	0	4	6	13

Histiological appearance in cases of complete response included foamy macrophages, frequently pigment laden and organized in nodule, fibrosis, and lymphocytic response. Cases with partial response variably showed the above features. Non-responders showed larger tumor (19 cm) or multiple node involvement (19/19) while the complete responders had tumors 3 cm or less and 0-1 nodes involvement. NC frequently induced "maturation" of the basaloid tumors that acquired increased amount of eosinophilic cytoplasm and an "squamoid appearance".

Conclusions: Our small group of TNT shows a low level of pathologic response that does not differ in pre and post menopausal age. This is most likely due to the large tumor size and nodes involvement in our patient population.

210 *HER2* Gene Amplification in Breast Carcinoma: A Methods Comparison Study between Silver In Situ Hybridization (SISH) and FISH

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Background: The importance of *HER2* status in breast cancer management has focused attention on the ability of clinical assays to correctly assign *HER2* amplification status. There is still no consensus on which is the best method for assessing *HER2* status. Disadvantages of FISH testing include longer time required for staining and scoring slides, requirements for specialized training and fluorescence microscopy, and impermanence of the slides. SISH is a fully automated assay providing permanent stained slides in six hours that are interpreted by conventional bright-field microsopy.

Design: 228 invasive breast carcinomas were analyzed for *HER2* gene amplification by SISH using bright-field microscopy following staining on an automated BenchMark® XT workstation [Ventana Medical System, Inc. (VMSI)] and by FISH using the PathVysion assay (Abbott Molecular/Vysis). *HER2* was quantified using the ratio of *HER2* to chromosome 17 (CHR17) signal counts using the conventional FDA scale (amplified if ratio ≥2, non-amplified if ratio <2); and using the ASCO/CAP reporting scheme (positive if ratio ≥2.2, negative if ratio <1.8, and equivocal if ratio 1.8 ≤ *HER2*/CHR17 ≤ 2.2). Discrepant cases between SISH and FISH were analyzed by immunohistochemistry (IHC) using the PATHWAY anti-HER2 (4B5) rabbit monoclonal antibody (VMSI) and scored as negative (0 or 1+), positive (3+) or equivocal (2+).

Results: Overall agreement between SISH and FISH was found in 214 (94%) of 228 cases using conventional criteria and 209 (95%) of 219 cases using ASCO/CAP result reporting scheme (with equivocal cases removed). Kappa statistics revealed excellent agreement between both methods using conventional and ASCO/CAP criteria (k=0.86 and k=0.90, respectively). When using the ASCO/CAP result reporting scheme, discrepancies between SISH and FISH occurred in 10 cases. Among nine cases categorized as *HER2* positive by FISH and negatine by SISH, 4 were scored negative by IHC, 3 were scored positive, and 2 were equivocal. The tenth discrepant case categorized as *HER2* positive by SISH and negative by FISH was negative by IHC.

Conclusions: The overall concordance between SISH and FISH was excellent. The SISH assay is fully automated and can be easily integrated into routine breast marker testing as an alternative to FISH for determination of *HER*2 status in breast carcinoma.

211 Silver In-Situ Hybridization (SISH) for Detection of *HER2* Amplification in Breast Carcinoma: International Inter-Observer Interpretive Reproducibility Study of 305 Cases

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Background: *HER2* SISH, a fully automated assay in which *HER2* gene status in breast carcinoma can be determined using bright field microscopy in only six hours, has emerged as an alternative to FISH. Preliminary studies have shown excellent concordance between SISH and FISH; studies of inter-observer interpretive reproducibility are lacking.

Design: Inter-observer interpretive reproducibility of *HER2* SISH was evaluated among eight pathologists in a consecutive series of 305 primary breast carcinomas for which FISH results were known. Two representative fields of invasive carcinoma were scored by each pathologist. Prior to scoring, a standardized tutorial study set was reviewed by all observers. Pathologists evaluated an H&E slide, a SISH slide stained for *HER2* using a repeat depleted DNP labeled probe, and a SISH slide stained for chromosome 17 (CHR17) using a DNP labeled centromeric oligonucleotide probe. *HER2* was quantified using the ratio of *HER2* to CHR17 signals using the conventional FDA scale (amplified if ratio ≥2, non-amplified if ratio <2); and using the ASCO/CAP reporting scheme (positive if ratio ≥2, negative if ratio <1.8, and equivocal if *HER2*/CHR17 ratio 1.8 - 2.2). Overall agreement and kappa were calculated for each observer versus other observers and versus reference *HER2* FISH.

Results: Excellent agreement between consensus SISH and corresponding FISH results (97%) using conventional scale and ASCO/CAP scale (93%) was observed (kappa 0.87 and 0.75, respectively). Interpretation of SISH results was highly reproducible among the eight pathologists, as overall agreement among observers and versus consensus SISH ranged from 94-98%. Agreement among observers and versus consensus FISH ranged from 91-96%.

Conclusions: *HER2* SISH is a rapid automated assay that uses bright field microscopy for slide interpretation. This enables pathologists to evaluate the slides within the context of tissue morphology. Results are highly concordant with FISH, and the interpretation of SISH results by pathologists is highly reproducible.

212 The Extent of Retraction Artifact Correlates with Lymphatic Vessel Density and VEGF-C Expression in Early Stage Breast Carcinoma and Predicts the Presence of Nodal Metastasis

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Background: Although the earliest feature of disseminated disease in breast cancer is regional lymph node involvement, little is known about the mechanisms whereby cancer cells interact with lymphatic endothelial cells and enter the lymphatic system. We have previously reported that the presence of extensive retraction artifact around

clusters of cells of breast carcinomas highly significantly correlates with lymphatic invasion and nodal meastasis and predicts poor survival independent of other prognostic factors. It was previously suggested that the clear spaces around the tumor cell nests are not just "artifacts" of fixation, but rather reflect an early stage of lymphatic invasion. In order to better understand the significance of the "retraction artifact" in relation to the mechanisms of lymphatic tumor spread we examined the correlation between the extent of retraction artifact and lymphatic vessel density and VEGF-C expression in a series of early stage breast carcinomas.

Design: Two hundred and fifty-six cases of stage pT1 and pT2 invasive breast carcinomas were selected. All H&E stained slides were reviewed and the diagnoses confirmed. The presence and extent of retraction artifact around tumor cells nests was determined based on all H&E slides. Lymphatic vessels were detected by D2-40 immunohistochemistry and the lymphatic vessel density (LVD) was determined using the hot-spot method. The expression of VEGF-C in the tumor cells was determined by immunohistochemistry and analyzed semiquantitatively on a 4-tiered scale.

Results: Breast carcinomas showing extensive retraction artifact (≥20% of tumor volume) were found to have significantly higher LVD levels and VEGF-C expression ($p<0.0001$, Kruskal-Wallis test) compared to tumors without this feature. The extent of retraction artifact in tumors highly significantly correlated with the level of LVD ($r=0.3545$, $p<0.0001$, Spearman test) and VEGF-C expression ($r=0.4163$, $p<0.0001$). High levels of LVD and VEGF-C expression significantly correlated with tumor size, grade, lymphatic invasion, nodal metastasis and poor outcome.

Conclusions: Our results suggest that the presence of extensive retraction artifact in breast carcinomas shows highly significant correlation with lymphangiogenesis (as determined by LVD and VEGF-C expression) and support the hypothesis that this phenomenon may represent an early stage of lymphatic invasion.

213 Differences in Prognostic Marker Expression in Female Breast Carcinoma with Brain Versus Bone Metastases

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Background: The ultimate clinical outcome of breast cancer patients depends on the development of systemic metastatic disease. Recent studies have shown that breast carcinoma metastatic to the brain is more likely to be ER and PR negative and to over-express HER-2/neu. We undertook a retrospective study of breast carcinoma with central nervous system or bone metastases to see whether histologic and/or tumor marker expression predict the pattern of brain or bone metastases.

Design: Our study population consists of 81 patients (38 with brain metastases and 43 with bone metastases) seen at our institution between 1995 and 2006. ER/PR were evaluated by immunohistochemistry (both Dako) and HER-2 status was evaluated by FISH (PathVysion, Vysis).

Results: The patients with bone metastases ranged in age from 34 to 73 years (mean of 54 years). Of the 43 cases with bone metastases, 33 (77%) were positive for ER and 29 (67%) were positive for PR. Of interest, HER-2/neu was overexpressed in only 1 (2%). Histologically, 29 (67%) were infiltrating ductal carcinoma and 14 (33%) were lobular carcinoma. The patients with brain metastases ranged in age from 34 to 84 years (mean of 58 years). Of the 38 cases with brain metastasis, 19 (50%) were positive for ER ($p=0.0122$) and 16 (42%) were positive for PR ($p=0.0221$). HER-2/neu was over expressed in 38 (29%) ($p=0.0008$). Histologically, all of these cases were ductal carcinoma ($p=0.0001$).

Conclusions: 1. In contrast to the breast cancer cases with bone metastases, the cases with brain metastases were more likely to be estrogen receptor negative, progesterone receptor negative, over-express HER-2/neu and be of ductal histologic type. 2. The patient group with brain metastases had a slightly older mean age than that with bone metastases, but the difference in age was not significant. These results suggest that patients with ER negative, PR negative, HER-2 overexpressing infiltrating ductal carcinomas are the subgroup of patients more likely to develop brain metastases. In addition, the relative age of the patients does not appear to be a factor in the pattern of metastatic growth. Finally, lobular histology seems to be associated with the development of bone but not brain metastases. These observations may aid in identifying a group of patients that may benefit from increased surveillance for the development of brain metastases.

214 Clinical, Morphologic and Immunophenotypic Characteristics of Breast Carcinomas with Lung Metastasis

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Background: Lung metastasis (LM) of breast carcinoma (BC) have high morbidity and mortality, and may precede the development of brain metastasis (BM). Information regarding BC with LM is limited, therefore we studied their clinical, morphologic and immuhophenotypic characteristics.

Design: We identified 97 patients (95 women, 2 men) treated for LM of BC at our institution (1980-2005). Slides and blocks of 49 BC were available for the study. Clinical data was extracted from the medical records. One pathologist (A.P.) assessed the morphology of all BC (including type, grade, DCIS, vascular invasion (LVI)). ER, PR, Her2, EGFR, Vimentin, CK5/6 and CK14 were tested on tissue microarrays; we used ER, PR and Her2 status of 1632 consecutive breast carcinoma diagnosed at our institution in 2006 as reference.

Results: BC occurred at median age of 48 y (range 24-76), with median size 11 mm (range 4-90; 28 unknown) and 44/86 nodal metastasis. LM developed at median age of 51 y (range 31-80), after 3 y median interval (range 0-24), and associated with bone (48), liver (44) and brain (22) metastasis. Morphologically, 46 tumors were ductal (42 NOS, 2 mixed ductal/lobular, 2 micropapillary) (6 grade 1, 25 grade 2, 15 grade 3) and 3 metaplastic. LVI was present in 32 cases. We noted more triple negative (TN), fewer ER(+) and fewer Her2(+) cases among BC with LM than in a reference group (Table 1).

ER, PR and Her2 status

	Reference Population		Breast Carcinoma	
	Positive/Total	%	Positive/Total	%
ER	1291/1632	79	28/48	58
PR	1045/1623	64	20/49	41
HER2	394/1561	25	8/49	16
Triple Negative	170/1561	11	17/49	35

15/48 patients for which BC immunoprofile was available developed BM, including 7/17 TN, 4/8 Her2(+) and 5/23 ER(+) and/or PR(+)/Her2(-), after 1.5 y median interval. Table 2 shows EGFR, Vimentin, CK5/6 and CK14 status in TN, Her2(+) and ER(+) and/or PR(+)/Her2(-) groups.

Immunophenotype in 49 BC

	Triple Negative (n=17)		Her2(+) (n=8)		ER(+) and/or PR(+)/Her2(-) (n=24)	
	Positive/Total	%	Positive/Total	%	Positive/Total	%
EGFR	13/17	76	0/8	0	3/23	13
Vimentin	12/17	71	1/8	13	2/23	8
CK 5/6	10/17	59	0/7	0	2/23	8
CK 14	8/15	53	1/8	13	0/24	0

Conclusions: Our results provide unique information on BC with LM. We report increased frequency of TN in this group, of which about 40% also developed BM. In addition, our data suggests that Her2(+) BC with LM, although fewer in number, are also likely to develop BM. These findings could help to predict tumor behavior and develop targeted treatment strategies.

215 Association of p4E-BP1 and EIF Family Factors with Grade of Malignancy and Metastasis in Breast Tumors. A Clinico-Pathological Study of 201 Cases

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Background: Cell signalling pathway is activated in nearly all breast tumors. In this pathway, many oncogenic signals can be activated including HER2, PI3K, PTEN, the ras-raf-ERKK cascades and others not well identified factors. Recently, we have proposed that in cell signaling there may be "funnel factors" which drive the oncogenic signal through ribosomes like the 4E-BP1 factor. The aim of this study was to make a complete evaluation of cell signalling factors including the receptors, the main biochemical pathways the 4EBP1 and EIF family factors in invasive breast carcinomas, metastasis and in situ tumors.

Design: We have studied 201 breast tumors, including 149 invasive carcinomas (42 with frozen tissue), 52 in situ carcinomas and 13 lymph node and pleural metastasis. The factors studied were HER2 growth factor receptor, the ras-raf-MAPK and the PI3K-AKT-mTOR pathways and the downstream factors p70S6, 4E-BP1, EIF4E and EIF4G.

Results: In the invasive cacinomas the percentage of HER2+ (3+) was of 87%. With pAKT and pMAPK no significant correlation was observed with stage or lymph node metastasis. With 4E-BP1, a strong nuclear and cytoplasmic positivity was observed in 69% of the in situ carcinomas (90% cribiform type and over 50% comedocarcinoma type). The 82% of invasive carcinomas showed a moderate-strong positivity. All the relapses and over 90% metastasis showed a strong cytoplasmic p4E-BP1 expression. The analysis of EIF family factors by IHQ and western-blot revealed overexpression of phosphoproteins in 81% (pEIF4E) and 25% (pEIF4G) cases, Moreover, those tumors with lymph node metastasis showed strong pEIF4E (81%) or p4E-BP1 (82%) expression.

Conclusions: Phosphorilation of 4E-BP1 and EIF4E factors associates with grade and lymph node metastasis regardless the HER2, PR, ER, pAKT, pMAPK expression. These results support that 4E-BP1 and the EIF family factors act as "funnel" factors in cell signalling and may be a novel therapeutical approach in most agressive tumors.

216 The mRNA and Protein Expression of IGF1R in Early Breast Carcinoma: Association with Local Recurrence

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Background: Insulin-like Growth Factor 1-Receptor (IGF1R) activation and/or overexpression have been demonstrated to lead cell proliferation and neoplastic transformation. Few data is available regarding its role in the development of metastasis and resistance to treatment. The aim of the present study was to investigate IGF1R as a potential risk factor for local recurrence (LR) in breast cancer (BC).

Design: Our series of 197 eligible BC patients in stage I-II treated with conservative surgery (CS) and radiation therapy (RT), included 33 (16.8%) who developed a LR and 16 (8.1%) with distant metastasis. The median follow-up was 99 months (range 16-234 months). Clinical and pathological features were assessed. Immunohistochemistry (IHC) for the receptor protein (alpha-IGF1R) (Neomarkers) and phosphorylation of IGF1R beta-subunit (Tyr1131/Insulin receptor Tyr1146) (Cell Signaling) was performed. Slides were scored semi-quantitatively based on staining proportion (0-100%) and intensity (1+, 2+, 3+) (range 0-300). In addition, we evaluated the IGF1R mRNA expression using quantitative real-time RT-PCR (qRT-PCR) in tissue samples from 85 primary BC (42 without recurrence, 31 with LR and 12 with distant metastasis) and 31 LR. The relationship between pathologic, IHC and qRT-PCR results were studied and correlated with the outcome.

Results: Tumors were predominantly <20mm in size (75.6%), of ductal type (98%), grade 3 (38%), without necrosis (61%), no vascular invasion (71.4%), with in situ component (84.2%) and negative margins of resection (>5mm) (75%). We found significantly lower levels of p-IGF1R in primary tumors with LR than in those with distant metastasis or did not recur (mean rank 49.3 vs 64.8 vs 75.2, respectively; $p=0.008$). However, there were no differences regarding the levels of IGF1R mRNA or alpha-IGF1R protein ($p>0.05$; Kruskall-Wallis test) or the pathologic features (all

p>0.05). Further comparison of the results with those from LR tissue showed only a positive correlation for alpha-IGF1R (Spearman correlation coefficient: r =0.432; p=0.019).
Conclusions: In the current series of BC patients treated with CS and RT, those with tumors showing lower p-IGF1R expression developed more frequently local recurrence. Therefore, our data indicate that p-IGF1R content is a favorable prognostic indicator. Supported by Grant FIS 03/1411.

217 Comparison of Clinicopathologic Features and Prognosis between 17 Conventional Malignant Phyllodes Tumors and 5 Malignant Phyllodes Tumors with Liposarcomatous Transformation of the Breast

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Background: Liposarcoma arising in a malignant phyllodes tumor (MPT) of the breast is uncommon, and is often associated with an adverse outcome. This study is to compare clinicopathologic features and natural history between conventional MPT (CMPT) and MPT with liposarcomatous transformation (MPTL). To the best of our knowledge, there has been no report of such comparison in the literature.
Design: 22 MPTs including 17 CMPTs and 5 MPTLs were identified from the surgical pathology files at our institution over a period of eleven years. The clinical information and pathologic features of the cases were reviewed retrospectively.
Results: Patient ages in the CMPT group ranged from 39 to 66 years (mean 54.6 years) and from 34 to 61 years (mean 52 years) in the MPTL group. CMPT tumor sizes ranged from 3 to 19.4 cm (mean 7.9 cm), while MPTL tumor sizes ranged from 10 to 25 cm (mean 15 cm). There is a borderline statistically significant difference in the tumor size between these two groups (p = 0.06). Of the 22 MPTs, 20 (90%) occurred in the right breast. The liposarcomatous elements in the MPTL group included myxoid liposarcoma with round cell differentiation and pleomorphic liposarcoma. There were no axillary lymph node metastases in either group. No tumors in either group expressed estrogen receptors, progesterone receptors, or had amplification of the Her2/neu oncogene. Two of 17 (12%) patients with CMPTs and 3 of 5 (60%) with MPTLs developed distant metastasis, mainly to the bone or lungs. There is a borderline statistically significant difference in the occurrence of distant metastasis between these two groups (p = 0.055). The remaining 15 cases of CMPTs were free of disease (1-7 years, mean 3.86) after excision; while the other 2 with MPTLs did not have follow-up information available. All 5 cases with distant metastases were high-grade tumors. The patients with metastatic tumors had no response to chemotherapy or radiation, and 3 out of the 5 patients died, while survival data for the other 2 patients was not available. However, the two patients did have widespread metastasis to multiple organs including the brain, heart, and soft tissues besides bone and lungs prior to being lost to follow-up.
Conclusions: In our case series, 23% of MPTs had liposarcomatous elements. MPTLs appeared to have a significantly higher risk to develop distant metastasis and a larger tumor size compared to CMPTs.

218 False Positive Sentinel Lymph Nodes in Breast Cancer Patients Caused by Benign Glandular Inclusions

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Background: Pathologic evaluation of sentinel lymph nodes (SLNs) provides a reliable means of predicting the status of the axillary lymph nodes in breast cancer patients. Recently, the concept of intranodal benign glandular inclusions (BGI) in SLNs has gained the attention of breast surgeons and pathologists, as their presence may be mistaken for metastatic carcinoma. Three cases with 4 SLNs containing the BGI are reported here, which were initially misdiagnosed as metastatic carcinoma. We hope this report will expand the literature on the BGI and heighten awareness of their existence and importance among pathologists.
Design: The 3 cases were identified at our institution in the past year. All patients had predominant high grade ductal carcinoma in situ along with small amounts of intermediate grade invasive ductal carcinoma. Ipsilateral SLN biopsies were performed. There were 4 SLNs identified, all contained benign appearing glandular elements, positive for cytokeratin. Immunohistochemistry for myoepithelial markers was performed on the SLNs. The morphological and immunophenotypic features of the BGI were reviewed and analyzed.
Results: The first case had a SLN with intranodal glandular elements adjacent a benign appearing squamous inclusion cyst. The 2nd case had a SLN with a single complex gland showing partial apocrine features. The 3rd case had 2 SLNs each with rare glands lined by bland columnar cells and surrounded by thin fibrous bands. Morphologically all the intranodal glandular elements did not resemble the corresponding invasive ductal carcinoma. All of the glandular elements in the 3 cases were positive for AE1/AE3 immunostains and were initially misdiagnosed as metastatic carcinoma. Additional immunohistochemistry for myoepithelial markers was performed on the first two cases and revealed smooth muscle myosin reactivity and scattered p63 positive nuclei, indicating the presence of myoepithelial cells. Based on both the morphology and immunohistochemistry, a diagnosis of BGI in the SLNs was established.
Conclusions: Our case series report indicates that comparison with morphology of primary breast carcinoma and using immunochemistry for myoepithelial markers are important ancillary tools in distinguishing BGI from metastatic carcinoma in SLNs. Common histopathologic features of the BGI include a lack of cytological atypia, occasional squamous and apocrine elements, and glandular structures primarily within the lymphoid stroma of the involved SLNs. A conservative approach to the management of such patients is recommended.

219 Most Basal-Like Breast Cancers Demonstrate an Rb Negative/p16 Diffuse Positive Immunophenotype

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Background: Basal-like carcinomas (BLC) of the breast, as defined by gene expression profiling, are high grade malignancies with a poor prognosis. By immunohistochemistry (IHC), BLC are estrogen receptor (ER), progesterone receptor (PR) and HER-2 negative ("triple-negative"), and typically express high-molecular-weight cytokeratins such as CK5/6. BLC often have a pushing border, minimal desmoplasia, a ribbon-like growth pattern with central necrosis, and focal squamous features. This morphology resembles that of specific human papilloma virus (HPV)-related poorly-differentiated carcinomas of the oropharynx, vulva, and penis. Inactivation of the Retinoblastoma (Rb) tumor suppressor by high risk HPV in the latter cancers leads to diffuse p16 labeling by IHC, since Rb normally suppresses p16 expression. The Rb/p16 immunophenotype of BLC of the breast has not been studied.
Design: We created tissue microarrays (TMAs) from 54 breast cancers which by IHC corresponded to specific types defined by expression profiling. These were luminal (ER+/HER-2-), HER-2+ (3+ score by IHC or amplification ratio>4 by FISH), and BLC (triple negative, strong CK5/6+). 18 triple negative but CK5/6- cancers (TNC) were also arrayed. The TMAs were analyzed by IHC for expression of p16, Rb, and Ki-67. HPV status was evaluated by in situ hybridization.
Results: The Rb negative/p16 diffuse positive immunophenotype (Rb-/p16+) was identified in 14 of 18 BLC and 11 of 18 TNC, but none of the HER-2+ or ER+ cancers (p<0.01). Elston Grade 3 BLC with the Rb-/p16+ phenotype had significantly higher Ki-67 indices (mean 72%) compared to Elston grade 3 HER-2+ cancers (mean 42%) (p<0.01). Among the combined 36 BLC and TNC, the 25 with the Rb-/p16+ phenotype had higher Ki-67 indices (mean 69%) compared to the 11 without the Rb-/p16+ phenotype (mean 53%) (p=0.03). None of these cancers contained HPV DNA.
Conclusions: Unlike other breast cancers, BLC frequently show an Rb-/p16+ phenotype, similar to the HPV-related cancers that they morphologically resemble, although BLC lack evidence of HPV infection. Inactivation of Rb likely promotes the high proliferative rate, and also may contribute to the histologic similarities between HPV-related cancers and BLC, though the inactivation occurs through different mechanisms. While BLC, as currently defined, are a heterogeneous group, the Rb-/p16+ phenotype may identify a more biologically homogeneous group which includes some triple negative, CK5/6- cases.

220 Inactivation of RUNX3 by Frequent Promoter Hypermethylation and Protein Mislocalization Constitute an Early Event in Breast Cancer Progression

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Background: We had previously established that inactivation of RUNX3 occurs by frequent promoter hypermethylation and protein mislocalization in invasive ductal carcinomas (IDC) of breast. Here, we analyze the protein expression and promoter methylation status of RUNX3 in ductal carcinoma in situ (DCIS) based on our hypothesis that inactivation of RUNX3 occurs early in breast carcinogenesis.
Design: The study cohort of 40 patients included 17 pure DCIS cases and 23 cases of DCIS with associated IDC (DCIS-IDC). The DCIS and IDC components of mixed cases were manually microdissected to permit separate evaluation. All the 63 samples including 17 pure DCIS, 23 samples each of DCIS and IDC of DCIS-IDC cases were analyzed for RUNX3 protein expression using R3-6E9 monoclonal antibody as well as promoter methylation status by methylation specific PCR.
Results: In contrast to the intense nuclear RUNX3 expression observed in matched normal breast tissue, 35 of 40 (88%) DCIS and 21 of 23 (91%) IDC samples exhibited downregulated RUNX3 expression in the form of negative or weak nuclear staining. Nuclear expression in DCIS and IDC were significantly lower than normal breast tissue (p<0.001). Promoter hypermethylation of RUNX3 was observed in 46 of the total 61 (75%) tumor samples including 30 of 40(75%) DCIS and 16 of 21(76%) IDC samples. Overall, promoter methylation correlated with loss of RUNX3 expression in 42 of 46 (91%) methylated samples. Mislocalized cytoplasmic expression accounted for RUNX3 inactivation in majority of DCIS and IDC samples independent of promoter methylation.
Conclusions: Our data suggest that RUNX3 hypermethylation and protein mislocalization constitute an early event in breast cancer progression.

221 Aplication of a Modified Gleason Score (MGS) in Infiltrating Ductal Carcinoma of the Breast

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Background: The most accepted system for grading infiltrating ductal carcinoma of breast (IDBC) is Scarff-Bloom-Richardson method (SBR). It has demonstrated that architectural and cellular parameters are related to prognosis. However, it has limitations: as it analyzes the proportion of tubular structures, nuclear grade, and number of mitoses per 10 HPFs, of these, only the first parameter can be evaluated accurately since the other two depend on tissue preservation, thickness of section and cellularity of the tumor. The Gleason score (GS) for prostatic carcinoma has proved to be an excellent prognostic and predictive factor. It is simple and reproducible; consists of histological assessment of the infiltration pattern and glandular characteristics seen under low power. GS has been applied worldwide and does not depend on nuclear details and mitotic count. Recently, a Gleason based system was applied to pancreatic carcinoma with a

good prognostic correlation. In this work, a modified Gleason system (MGS) applied to IDBC was compared with the SBR system for inter and intra-observer reproducibility, as well as its value as prognostic factor.
Design: 99 cases of IDBC were analyzed, SBR histological grade was compared with a MGS score (MGSS). All cases were reviewed twice by 3 observers with different degree of professional practice (3,7 and 30 years) to determine intra and inter-observer reproducibility. MGS consisted in 3 basic patterns: tubular (Grade 1), cribriform or trabecular (Grade 2), and solid or in cords (Grade 3). The sum of the 2 predominant patterns yielded the MGSS. Tumors with MGSS $\leq$4 were considered as low grade (LG), whereas those with a MGSS >4 were classified as high grade. SBR and MGSS were correlated with clinical outcome. Statistical analysis was done to determine inter and intraobserver reproducibility and survival curves.
Results: Global disease-free survival (DFS) at 5 and 10 years was 85% and 75% respectively. Lymph node status ($p<0.0001$), pathological stage ($p<0.0001$) and grade obtained with the MGS were better correlated with DFS, with similar values in all three observers ($p<0.04$, $p<0.036$ and $p<0.05$). No patient with a LG tumor obtained by MGS recurred. Grade obtained by SBR did not correlate with DFS ($p = 0.89$).
Conclusions: The MGS applied to IDBC NOS is easy and objective, based only in architectural patterns, its application is simple and independent of the observer experience (interobserver concordance 72%-81%), and has a good correlation with DFS ($p<0.036$).

222 Comparative Genomic Hybridization Identifies High Frequency Chromosomal Alterations in Adenoid Cystic Carcinoma of the Breast

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Background: Adenoid cystic carcinoma of the breast (Br-ACC) morphologically resembles ACC of salivary gland (Sg-ACC) but behaves in a distinctly less aggressive manner. Reported cytogenetic abnormalities in Sg-ACC have included frequent loss of 12q and 6q and gain of 16p and 22q. By contrast, cytogenetic abnormalities have not been studied in Br-ACC. We used high resolution comparative genomic hybridization (CGH) to determine whether Br-ACC harbors similar and/or unique abnormalities compared to those reported in Sg-ACC.
Design: Genomic DNA was purified from formalin fixed, paraffin embedded tissue from 9 cases of Br-ACC. Samples were hybridized to the human whole genome 32K Tiled BAC array comprising 32,060 clones. We acquired 16 bit DAPI, Cy3 and Cy5 images that were used to calculate various measurement parameters including $\log_2$ ratios of the total integrated Cy3 and Cy5 intensities for each spot. Clone identities and mapping information files were used to plot the data relative to the position of the BACs using the draft human genome sequence (http://genome.ucsc.edu). Results were compared to published cytogenetic data on Sg-ACC.
Results: Overall, Br-ACC showed a high fraction of genomic alteration (mean fraction of genome altered for the series was 0.218). Similar to Sg-ACC, loss of 12q24 was seen in 3/9 Br-ACC and gain of 22q in 7/9 Br-ACC. Alterations unique to Br-ACC included: 8/9 cases had gain of 19p13.3 (loci for CNN2 calponin, STK11, and KISS1R metastasis suppressor protein receptor in breast cancer). Six cases had gains of 11q13 (CCND1), 12p13, 16q24, 19p13. Loss in 8/9 cases was found for 9p13 10p11, 14q11. In 7/9 cases there was loss of 1p12 (BCL-9), 2p11, 5q13. In 6/9 cases there was loss of 1p36 (NOTCH2), 2q21, 4p11, 7p11, 8p23, 10q11 (RET), 15p11 (lost in estrogen receptor negative breast cancer) and 21p11.
Conclusions: Despite morphological similarities, Br-ACC harbored unique chromosomal alterations that differentiated it from Sg-ACC. Several of these alterations were found at high frequency and suggest loci for potential genes that may play a role in the pathogenesis of this rare breast tumor.

223 Value of Mammaglobin Immunostain in Estrogen Receptor Negative Metastatic Breast Carcinomas: A Comparative Analysis of 61 Cases

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Background: Identifying the site of origin of metastatic adenocarcinomas in biopsy specimens in daily pathology practice relies on clinical history, morphology and immunohistochemistry. Breast carcinomas are among the most common primary tumors to metastasize, but might be histologically indistinguishable from other primary carcinomas. Mammaglobin is increasingly being used as an initial marker in conjunction with Cytokeratin 7 (CK7), Estrogen Receptor (ER) and Gross Cystic Disease Fluid Protein-15 (GCDFP) to characterize breast carcinoma. In this study, we aim to determine the positive predictive value of adding Mammaglobin to the initial panel of CK7, ER and GCDFP to identify metastatic breast carcinomas.
Design: We evaluated the above-mentioned immunostaining characteristics in 28 cases of metastatic adenocarcinomas detected in biopsy specimens from various sites in patients with a clinical history of primary breast carcinomas (9 livers, 6 lymph nodes, 4 visceral soft tissues, 3 bone, 3 neck soft tissue, 2 chest wall, 1 brain). We compared the expression of Mammaglobin, CK7, ER and GCDFP to tissue micro arrays from 33 representative sections of breast-confined invasive carcinomas.
Results:

Comparison of Positive Immunostaining Results

Stain	Metastases	Breast confined carcinomas
Mammaglobin	86% (24)	49% (16)
ER	57% (16)	70% (23)
GCDFP	18% (5)	9% (3)
CK7	93% (26)	91% (30)

Staining in Estrogen Receptor negative tumors

Stain	Metastases	Breast confined carcinomas
Mammaglobin +	75% (9)	30% (3)
GCDFP +	10% (1)	10% (1)
Mammaglobin +, GCDFP -	89% (8)	100% (3)

There is no statistically significant difference in the Mammaglobin immunostaining properties among metastases and breast-confined carcinomas that are ER negative (Fischer exact test, p-value= 0.09). Additionally, only 11% (1) of ER negative, Mammaglobin positive cases stained for GCDFP.
Conclusions: The Mammaglobin immunostaining characteristics of ER negative breast carcinomas is independent of the tumor being breast-confined or a metastasis. Mammaglobin is more sensitive than GCDFP, especially in ER negative cases. These results support the additional value of Mammaglobin as part of the marker panel along with CK7, ER and GCDFP in identifying metastatic adenocarcinomas as originating from primary breast carcinomas, particularly in characterizing ER negative cases.

224 Is Acinic Cell Carcinoma a Variant of Secretory Carcinoma? A FISH Study Using *ETV6* 'Split Apart' Probes

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Background: Acinic cell carcinomas (ACCs) and secretory carcinomas (SCs) of the breast are rare, low grade malignancies that preferentially affect young female patients. Owing to the morphological and immunohistochemical similarities between these lesions, they have been proposed to be two morphological variants of the same entity. It has been recently been demonstrated that SCs of the breast consistently harbour the t(12;15) *ETV6-NTRK3* translocation. We hypothesised that if ACCs were variants of SCs, it would be reasonable to expect that ACCs would also harbour the t(12;15) chromosomal translocation.
Design: We investigated the presence of *ETV6* rearrangements in 3 SCs and 6 ACCs using the *ETV6* FISH DNA Probe Split Signal (Dako, Glostrup, Denmark). Cases were considered as harbouring an *ETV6* gene rearrangement if >10% nuclei displayed 'split apart signals' (i.e. red and green signals were separated by a distance greater than the size of two hybridisation signals).
Results: Whilst the three SCs displayed *ETV6* split apart signals in >10% of the neoplastic cells, no ACC showed any definite evidence of *ETV6* gene rearrangement. Interestingly, additional copies of chromosome 12p were identified in the non-modal population of two acinic cell carcinomas. In addition, one of the secretory carcinomas harboured additional copies of the probe telomeric to the breakpoint on 12p.
Conclusions: Based on the lack of *ETV6* rearrangements in ACCs, our results strongly support the concept that secretory carcinomas and acinic cell carcinomas are distinct entities and should be recorded separately in breast cancer taxonomy schemes.

225 *ESR1* Gene Amplification: A Common Phenomenon?

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Background: It has been recently reported that 20.6% of breast cancers harbour *ESR1* gene amplification. *ESR1* amplification correlated with ER expression, 0.6% of the amplified cases failed to show expression of the gene product. It has also been suggested that *ESR1* amplification could help identify a subgroup of breast cancer patients that would benefit most from endocrine therapy. The aim of this study was to investigate the prevalence of *ESR1* gene amplification in a cohort of 245 invasive breast cancers.
Design: We investigated the prevalence of this finding in a cohort of 245 invasive breast carcinomas, 80.1% of which were ER-positive, arranged in a tissue microarray containing representative replicate 0.6 mm tumour tissue cores. Sections of the tissue microarrays were subjected to chromogenic *in situ* hybridisation (CISH) with in house generated probes for the *ESR1* gene composed of 2 bacterial artificial chromosomes (RP11-655I21 and RP11-517B22). Amplified cases were defined as those with >50% of neoplastic cells harbouring >5 *ESR1* gene copies/ nucleus or large *ESR1* gene clusters. Cases defined as amplified by CISH were subjected to fluorescent *in situ* hybridisation to confirm the presence of *ESR1* amplification.
Results: Out of 245 cases, 148 rendered optimal CISH results. Only 2 cases (1.35%) harboured large *ESR1* gene clusters. These 2 cases and 8 additional CISH non-amplified cases were subjected to FISH, which confirmed the results of CISH analysis.
Conclusions: Although we have identified *ESR1* gene amplification, this phenomenon was substantially less prevalent than previously described. Our findings do not support *ESR1* amplification being instrumental in defining a subtype of primary breast cancers optimally suited for hormonal therapy.

226 Is ER/HER2 Testing Indicated in All Histologic Types of Breast Cancer?

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Background: The phenotypic expression of estrogen receptor (ER) and HER2 have been shown to be consistent in certain histologic types and grades of mammary carcinoma. With the availability of markers for several morphologic variants of breast cancer, it is now possible to classify these tumors more objectively. The purpose of this study was to evaluate the ER/HER2 profile of immunophenotypically confirmed subtypes of human mammary carcinomas.
Design: Core needle or excisional biopsies from one thousand consecutive cases of untreated invasive breast carcinomas were evaluated for the immunohistochemical expression of ER and HER2. The staining results for HER2 were scored as negative (0 and 1+), inconclusive (2+) and positive (3+). The inconclusive HER2 cases were triaged for HER2-FISH, and recorded as positive when amplified. Based on histology alone, tumors were classified into ductal and non-ductal categories. In the ductal group the nuclear grades were recorded as low, intermediate and high. In those cases that morphologically a non-ductal phenotype was suspected by one or both reviewing

pathologists, the following markers were used for confirmation: E-Cadherin for lobular carcinoma, p63 for metaplastic carcinoma, HLA-DR for medullary carcinoma and androgen receptor (AR) and GCDFP-15 for apocrine carcinoma.
Results: Based on histomorphology and immunophenotype, tumors were classified as 911 cases of ductal and 89 cases of non-ductal carcinoma. Of the non-ductal type, 40 cases were lobular (E-cadherin-negative), 22 metaplastic (p63-positive), 22 medullary (HLA-DR-positive), and 5 apocrine (AR-GCDFP-15 positive). Ductal carcinomas were separated into no special types (DC-NST, n=873), tubular (n=17), colloid (n=13), and papillary (n=8). All low nuclear grade DC-NST, and all tubular, papillary, and colloid carcinomas, as well as all lobular carcinomas were positive for ER. Conversely, all metaplastic, medullary and apocrine carcinomas were negative for ER. HER2 expression was limited to intermediate grade (94/610 or 15.4%) and high grade (47/154 or 30.52%) DC-NST carcinomas. All low nuclear grade DC-NST, tubular, papillary, and colloid, as well as all non-ductal carcinomas were negative for HER2.
Conclusions: In immunophenotypically confirmed histologic types of mammary carcinoma, the expression (or lack of expression) of ER and HER2 is consistent and predictable. Our results along with previously reported observations raise an interesting question: should ER and HER2 testing be limited to ductal carcinomas of no special type?

227 Novel Non Biotin Polymeric Immunodetection Systems Provide Stronger Signal without Background in Estrogen Receptor Evaluation of Breast Carcinomas

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Background: Immunohistochemistry (IHC) is a multistep diagnostic test highly dependent on appropriate tissue processing and reagents, including the immunodetection system. A novel generation of detection systems using a non-biotin polymeric technology (NBP) has been released to avoid problems related to endogenous biotin present in the streptavidin-biotin systems (SAB). **Aim:** We compared the new NBP and the SAB systems to evaluate estrogen receptor (ER) in breast carcinomas.
Design: Serial sections from a tissue microarray containing 300 invasive breast carcinoma samples of formalin-fixed paraffin- embedded tumors were immunostained for ER with SP1 rabbit monoclonal antibody (LabVision). Four different detection systems were used, including 2 NBP of second generation (Novolink Polymer, Visionbiosystems™; Super Sensitive Polymer–HRP, Biogenex™); the first generation NBP (EnVision+, Dako™), and one SAB system (LSAB+, Dako™). The dilution of the primary antibody SP1 was 1:300 and the incubation time 30 minutes. Reagents supplied by NBP immunodetection systems kit were used for antigen retrieval, peroxide block and IHC wash. All procedures and incubation times followed instructions supplied with the reagents. The immunostains were evaluated using a light microscope and Allred's scoring system. Cases were considered positive when the proportion score was >2.
Results: There was no difference in the number of positive cases comparing the NBP and SAB. However staining intensity of positive cases developed with the NBP was significantly higher than those cases developed with SAB ($p<0,05$). All 3 NBP detection systems provided similar scores of intensity without background or non-specific cytoplasmatic staining. Background was highly observed in reactions developed with the SAB ($p<0,05$).
Conclusions: The non biotin polymeric systems provide stronger and sharper immunohistochemistry signal, without background or non-specific cytoplasmic staining compared to streptavidin biotin system.

Specification, type, and supplier of each immunodetection system

Immunodetection System	Type	Supplier
EnVision +	Non-biotin polymer, first generation	Dako
NovoLink Polymer	Non-biotin polymer, second generation	Visionbiosystems
Super Sensitive HRP	Non-biotin polymer, second generation	Biogenex
LSAB +	Streptavidin-biotin complex	Dako

228 Histologic Characterization of In-Situ Carcinoma in BRCA1 and BRCA2 Mutation Carriers without Invasive Disease: A Population-Based Study

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Background: Invasive breast cancers in women with BRCA1 and BRCA2 germline mutations have distinct histologic, immunohistologic and molecular phenotypes. Despite recent advances in our understanding of invasive cancer in the BRCA patient population, information is limited concerning the incidence and phenotype of in situ cancers. Since invasive cancers in BRCA germline mutation carriers are different from sporadic carcinoma, it is likely that precursor lesions associated with BRCA cancer are also different from those in sporadic cancer. Identification of these differences would enhance screening protocols for BRCA patients and provide additional insight into the development of BRCA-associated invasive carcinoma.
Design: Two sites within the International Collaborative Familial Breast Cancer Registry (The Northern California Cancer Registry (NCCR) and the Ontario Familial Breast Cancer Registry (OFBCR)) have accrued women with premalignant (non-invasive) breast cancers into a prospective population-basted cohort study which includes demographic, genotyping, and histopathologic data on probands. Histologic features of in-situ carcinoma recorded include the nuclear grade, presence of necrosis and predominent growth pattern. Data was reviewed and correlated with BRCA1 and BRCA2 mutation status. The pathology of BRCA1/2 DCIS was compared with the control population.
Results: Of 165 subjects presenting with in situ carcinoma only, germline BRCA1 and BRCA2 mutations were identified in 3.6% (n=6) and 8.5% (n=14), respectively. One of the BRCA2 carriers also had a mutation for BRCA1. The pathologic characteristics of DCIS in BRCA1 and BRCA2 carriers are summarized in Table 1. Four LCIS only cases were also identified within the study groups (3 BRCA2, 1 BRCA1).

	BRCA1	BRCA2	Controls
Age, mean (yr)	47.5	47	48.7
Necrosis (%)	100	90.9	72
Nuc Grade 1 (%)	0	18.2	23
Nuc Grade 2 (%)	50	27.3	33
Nuc Grade 3 (%)	50	54.5	44
Nuc Grade 2/3 (%)	100	75	77
Solid*	100	72.7	28.5
Cribriform	0	18.2	35

*Primary pattern contains, but not exclusively, solid
Conclusions: DCIS in BRCA1/2 mutation carriers trends towards higher nuclear grade and necrosis relative to sporadic DCIS. This trend is more pronounced in BRCA1 mutation carriers. DCIS in BRCA1/2 carriers may have distinct phenotypes, although numbers are limited.

229 Comparative Evaluation of the HER-2 Gene Status in Breast Carcinoma with Immunohistochemistry, Chromogenic In Situ Hybridization and Real-Time Quantitative PCR

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Background: The human epidermal growth factor receptor-2 (HER-2) proto-oncogene, located on chromosome 17, encodes a protein with tyrosine kinase activity. Amplification of the gene has been reported in 15-30% of invasive breast carcinomas and correlated with a poor prognostic. The clinical implication of HER-2 testing is the selection of patients for treatment with anti-EGFR monoclonal antibody drugs developed against HER-2 product. The purpose of this study was to compare the respective efficiency of three methods for HER-2 measurement.
Design: The study included 43 patients with 40 invasive breast carcinomas and 3 pure ductal in situ carcinomas. Forty one cases were fixed in alcohol-formalin-acetic acid (AFA) and 2 cases in neutral buffered formalin. All cases were analyzed by immunohistochemistry with antibody Dako485®, Chromogenic in situ Hybridization with digoxigenin-labeled-HER-2 DNA probe (Zymed®) and Real-Time Quantitative PCR using Taqman® probes on the Rotor Gene 6000, Corbett®.
Results: The concordance rate between the three methods was 90%, and coefficient κ=0, 84 (IC 0,64-1,04). We found a perfect concordance between genomic methods, CISH and Real-Time PCR with coefficient κ=1. No false negative case were found with immunohistochemical analysis, but 5 cases scoring 3+ with Herceptest® were not found amplified with genomic techniques. Three cases could not be evaluated with CISH, attributed to AFA induced DNA degradation, but were retrieved with RT-PCR. Intra-ductal component from invasive carcinomas did not induce RT-PCR false positive.
Conclusions: Chromogenic in situ hybridization is a sensitive and specific method to determine the HER-2 status of surgical breast carcinomas, especially for testing cases graded 2+ using immunohistochemistry. The results obtained with Real-Time Quantitative PCR match perfectly with those obtained with CISH. Real-Time PCR is an economical, fast, accurate and effective approach, that could be proposed as an alternative to immunohistochemistry and CISH for establishing HER-2 status in breast carcinomas.

230 WT-1 Expression in Breast Carcinomas with Mucinous Morphology

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Background: Most immunohistochemical studies of primary breast carcinomas have demonstrated only rare expression of WT-1. Recent studies, however, have indicated that strong WT-1 expression is sometimes seen in the micropapillary subtype. The aim of our study was to assess WT-1 expression in mucinous breast carcinomas.
Design: Twenty-four primary invasive breast carcinomas with mucinous features were retrieved from the case files at our institution. These were further subdivided into "pure mucinous" carcinomas (at least 90% of the tumour was comprised of cells with a low nuclear grade floating in mucin pools, 14 cases) versus invasive ductal carcinoma with mucinous features (tumours with extracellular mucin, but too high a nuclear grade for classification as a mucinous carcinoma, 10 cases). In addition, thirty-four consecutive cases of invasive duct carcinoma, not otherwise specified (NOS) were reviewed for comparison. Immunohistochemical staining for WT-1 was performed on all cases.
Results: Moderate to strong nuclear WT-1 staining was seen in 16 out of 24 cases of breast carcinoma with mucinous morphology (67%). Pure mucinous carcinomas were positive for WT-1 in 10 out of 14 (71%) of cases, while 6 out of 10 cases (60%) of invasive duct carcinomas with mucinous features were positive. Only 2 out of 34 cases (6%) of invasive duct carcinoma, NOS showed nuclear positivity for WT-1.
Conclusions: A significant percentage of breast carcinomas with mucinous features demonstrate expression of WT-1. In the setting of a metastatic mucinous carcinoma, nuclear staining for WT-1 may be helpful in distinguishing a breast primary from mucinous ovarian or colorectal primaries, which are negative for nuclear staining.

231 Analysis of Fixative Type (Formalin vs Penfix) and Fixation Time for Hormone Receptors and Her 2 Immunohistochemical: A Validation Study

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Background: Proper fixation for immunohistochemical analysis of estrogen (ER) and progesterone (PR) hormone receptors and Her 2 is the first critical step to assure quality and consistent results for patient care. There is a dearth of literature on fixation for these predictive/prognostic markers, although recent recommendations indicate an

optimum fixative exposure time of 6-48 hours in 10% neutral buffered formalin (NBF) Penfix, an alcoholic formalin, is commonly used as an aid in fatty tissue processing to enhance proper sectioning. In this study, the effects on hormone receptor and Her 2 IHC expression with utilization of an initial pre-established NBF or Penfix fixation exposure time followed by standard tissue processing in NBF was analyzed. Results of P53 immunohistochemical staining in non-neoplastic tissue were monitored as an indicator of fixation quality.

Design: Six breast carcinomas were harvested fresh, divided, and submitted into10% NBF or Penfix followed by 10% NBF. The initial fixation exposure times for each case were 6, 8, 24, 72 and 126 hours. The 8-hour sample was reference result for all fixation times. Immunostaining for ER (6F11), PR (1A6) and Her 2 (CB11 and 4B5) were preformed on the Benchmark XT according to established protocols. Hormone receptors were semi-quantitated using the H-Score, and Her 2 was interpreted according to CAP-ASCO guidelines. A Student T Test was used to compare H Scores.

Results: One case had tissue at only the 126 hours and showed a decrease of the Her 2 (CB11) expression from 3+ to 2+. There were no significant differences in the ER/PR/P53 H Scores for 6, 8, 24 or 72 hours fixative exposure times. The 8hour time point from one case was not analyzed due to absence of invasive carcinoma.

Conclusions: (1) Exposure of breast specimens to Penfix prior to formalin exposure does not affect clinical results of these IHC tests. (2) ER/PR and Her2 show robust results from 6-72 hours of formalin exposure. (3) ER/PR remained robust at 126 hours of fixation, but the Her2 (CB11) began to decrease in intensity. (4) It is important to validate any formalin-variant fixatives and processing methods to assure quality results.

232 Peritumoral Lymphatic Microvessel Density and Lymphovascular Invasion Detected by D2-40 as Prognostic Markers in Invasive Micropapillary Breast Carcinoma

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Background: Invasive micropapillary carcinoma (IMP) is characterized by high tendency for lymphovascular invasion and lymph node (LN) metastases. However, there is limited data evaluating the significance of lymphatic microvessel density (LMD) as a prognostic marker in these patients. In this study, we investigated LMD and lymphovascular invasion, detected by D2-40, as predictive markers for the risk of LN metastases and its relation to other prognostic parameters in IMP patients.

Design: Fifty cases of IMP treated with lumpectomy and LN dissection were reviewed. We also included 40 cases of invasive ductal carcinoma, NOS, as a control group. Cases were stained for CD31 and D2-40. Positive stained microvessels (MV) were counted in densely lymphatic/vascular foci (hot spots) at 40x (=0.17 mm^2) in each specimen by 2 pathologists. Results were expressed as the highest number of MV count identified within any single field and correlated with other clinicopathologic prognostic parameters.

Results: In micropapillary group, there was a positive correlation of both D2-40 and CD31 MV count with LN metastases (r=0.41, 0.36), and pathologic stage (r=0.39, 0.42). Only peritumoral D2-40 LMD correlated significantly with the presence of lymphovascular invasion in general, distant metastases and Her2/neu status (r=0.4, 0.3, 0.5; respectively). Lymphovascular invasion detected by D2-40 showed significant correlation with LN status (r=0.73), tumor size (r=0.46), distant metastases (r=0.5), and Her/neu2 status (r=0.43).

	Micropapillary group	NOS group
PT-LMD	13±5	9±7*
IT-LMD	5±6 (in 32%)	4±6 (50%)
CD31 MV	29±10	20±13*
LVI D2-40	28/50 (56%)	15/40 (37%)*
LVI CD31	21/50 (42%)	10/40 (25%)
LVI H&E	15/50 (30%)	4/40 (10%)
LN metastases	27/50 (54%)	13/40 (33%)*

PT= peritumoral, IT= intratumoral, LMD= lymphatic microvessel density, LVI= lymphovascular invasion, LN= lymph node, * statistically significant

Conclusions: Our study showed that both LMD and lymphovascular invasion play an important role in the progression of micropapillary breast carcinoma. In comparison to NOS group, IMP showed a significant higher lymphovascular invasion and peritumoral LMD, which may explain their aggressive behavior. Peritumoral LMD detected by D2-40 can be used as prognostic predictor since it demonstrated significant correlation with LN metastases, tumor stage, lymphovascular invasion, distant metastases and Her2/neu status.

233 HER2 Heterogeneity in Primary Breast Cancers: A Tissue Micro-Array Analysis of FISH and IHC

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Background: The concordance rate for detection of *HER2* (*ERBB2*) gene amplification by FISH between core and excisional biopsies of primary breast carcinomas (BrCa) is reported to be close to 100%. However, HER2 expression/amplification discordance between primary and metastatic breast cancer (BrCa) has been reported in up to 26% of the cases suggestive of progression of tumor phenotype or selection of a subpopulation within tumor. We performed *HER2* FISH and IHC on primary BrCa tissue microarrays (TMA) to identify intratumoral heterogeneity, which may be "diluted" and not recorded in evaluating whole sections of primary BrCa.

Design: 1 mm TMAs were constructed from primary BrCa. Cases with 2 or more cores were used for evaluation of discordance within *HER2* FISH and IHC groups (n=105 and n=110, respectively). Cases with only one core (n=149) were also included when evaluating discordance between different tests (i.e., FISH and IHC). *HER2* FISH was evaluated by scoring 20 cells in each core; the cores were categorized as amplified (A) if the *HER2*/CEP 17 ratio was ≥2 and not amplified (NA) if <2. A core was categorized as discordant if its ratio changed from A to NA, or vice versa. IHC cores were considered discordant when scores changed from 0/1+ to 2/3+ or from 2+ to 3+ or vice versa. 2+ IHC was not scored as 'discordant' irrespective of FISH result (A or NA) when assessing FISH-IHC discordance as, in clinical practice, 2+ IHC are not just scored "positive" or "negative" but reflex FISH is performed for all.

Results: 4/105 (3.8 %) of cases with ≥ 2 cores show discordance in FISH results (2 cores: 2/62, (3.2%); 3 cores: 2/39 (5.1%); 4 cores: 0/4 (0%).

	Case 1 Cores	Case 2 Cores	Case 3 Cores	Case 4 Cores
FISH	NA / A	A / NA / NA	NA / A	A / A / NA
IHC	0 / 2	0 / 0 / 0	0 / 0	3 / 3 / 3

Discordance in IHC testing was seen in 18/110 (16.4%) of the cases (2 cores: 10/65 (15.4%); 3 cores: 8/41 (19.5%); 4 cores: 0/4 (0%)). Discordance between FISH and IHC testing was seen in 5/149 (3.4%) cases.

Conclusions: Intratumoral heterogeneity for *HER2* amplification by FISH within the primary BrCa is not common and may be responsible for discordant results between primary and metastases if the amplified clone preferentially metastasizes. Discordance between FISH and IHC is low since reflex FISH testing is done for all 2+ cases clinically.

234 Expression of the Rho GTPase Cdc42 in Triple-Negative Breast Cancers (TNBCs), Including Metaplastic Carcinomas

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Background: TNBCs, including metaplastic breast carcinomas, are a group of poor prognosis breast tumors lacking expression of ER/PR/HER2. Many are basal-like tumors that frequently express EGFR and basal cytokeratins. TNBCs currently lack targeted therapies. Rho GTPases, including Cdc42, are Ras homologues, some of which appear to play a role in malignant transformation and metastasis. We previously demonstrated that activation of the cell adhesion molecule β4 integrin in a cell line model of TNBCs resulted in increased gene expression of Cdc42. For the current study, we investigated expression levels of Cdc42 in TNBCs, including a subgroup of metaplastic breast cancers, in order to validate our previous in-vitro findings and to further characterize the biomarker profile of this aggressive form of breast cancer.

Design: Whole tissue sections from 51 TNBCs, including 25 metaplastic carcinomas, were studied. These tumors were previously assayed for multiple markers including β4 integrin. For the current study, Cdc42 was detected with a monoclonal antibody and automated immunohistochemical methods. The staining intensity of Cdc42 was stratified as: 0 (negative), 1+ (faint), 2+ (moderate), 3+ (strong). A tumor was considered positive for Cdc42 if more than 10% tumoral expression was observed with at least 2+ staining intensity.

Results: Staining for Cdc42 was adequate for evaluation in 51 cases. We found that 33/51 TNBCs (65%) were positive for Cdc42 expression using the criteria defined above. Whereas 13/26 cases of non-metaplastic TNBCs (50%) had Cdc42 expression, 20/25 cases of metaplastic breast carcinoma (80%) were positive for Cdc42 expression. The increased rate of Cdc42 expression seen in metaplastic breast cancer cases compared to the non-metaplastic TNBCs was statistically significant (P=0.025). Moreover, in the metaplastic subgroup, Cdc42 was significantly associated with β4 integrin expression (P=0.039), supporting our in-vitro findings showing increased Cdc42 expression following activation of β4 integrin.

Conclusions: Our results show significant levels of Cdc42 expression in TNBCs, particularly in metaplastic breast carcinomas. The high expression rates observed for metaplastic cancer cases suggests that Cdc42 may play a particularly important role in the pathogenesis and behavior of these tumors.

235 Use of pHH3, Ki-67 and Survivin Immunoreactivity in the Evaluation of Fibroadenoma and Phyllodes Tumors of Breast

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Background: Accurate categorization of phyllodes tumors (PT) and their differentiation from fibroadenoma (FA) can be sometimes challenging especially in core needle biopsies. Benign, low grade or borderline and malignant PT differ from each other in their clinical course with regards to local recurrence and metastasis as well as surgical management.

Design: Anti-phosphohistone H3 (pHH3), Ki-67 (MIB-1 antibody) and survivin immunostaining was performed on formalin fixed, paraffin embedded sections of 30 cases (20 cases of PT and 10 cases of FA). PT were classified as benign (8 cases; mean age 35 years), low grade (7 cases; mean age 37 years) and malignant (5 cases; mean age 53 years) based on mitotic count, stromal cellularity and pleomorphism. For pHH3, mitotic count was evaluated per 10 high power fields. For Ki-67 and survivin, one thousand cells were counted randomly and the labeling index was recorded as the percentage of positive nuclei.

Results: pHH3 immunostaining aided in highlighting the mitotic figures and classification of PT. The Ki-67 labeling index was 1.1% for cases of FA (range 0.01 to 2.4%), 0.8% in benign PT (range 0.2 to 2.2%), 7.6% in low grade PT (range 4.2 to 11.8 %) and 29.3% in malignant PT (range 15.1 to 41.9%). There was no cytoplasmic staining of survivin. Survivin nuclear staining index was 0.1% for cases of FA (range 0 to 0.3%), 0.2% in benign PT (range 0 to 0.6%), 0.4% in low PT (range 0.2 to 0.8 %) and 14.3% in malignant PT (range 11.2 to 17.5%).

Conclusions: Precise diagnosis of PT is important as it influences the surgical management and clinical followup. Our findings support the diagnostic utility of Ki-67 in differentiating FA and benign PT from low grade and malignant PT. In addition we demonstrate the use of survivin in differentiating low grade from malignant PT.

236 A Ten Year Review of 837 Cases of Lobular Neoplasia: Association with the Spectrum of Ductal Neoplasia

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Background: There has been recent attention drawn to the association between flat epithelial atypia (FEA) and lobular neoplasia (LN). The association of LN and ductal neoplasia (DN) is not unique to FEA and is also seen with other forms of DN. However, the actual incidence is not known.

Design: We reviewed the reports of all cases diagnosed with LN that included atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS) and invasive lobular carcinoma (ILC) for a ten-year period (1995 and 2004). A total of 837 cases of LN were identified and reviewed for the concurrent presence of DN i.e. FEA, atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) and its special subtypes such as tubular and mucinous carcinoma. In cases with more than one type of lesion, the higher or worse lesion was regarded as the index lesion for our study. The incidence and associations were calculated.

Results: ILC alone was seen in 29.03% (n=243), associated with ADH in 1.08 % (n=9), DCIS in 5.37% (n=45) and with IDC in 1.67% (n=14). LCIS alone was seen in 9.44% (n=79), associated with FEA in 0.12% (n=1), ADH in 5.14 % (n=43), DCIS in 6.57% (n=55) and with IDC in 8.60% (n=72). ALH alone was seen in 14.81 % (n=124), associated with FEA in 0.84 % (n=7), ADH in 8.00 % (n=67), DCIS in 4.54 % (n=38) and with IDC in 4.78 % (n=40). 45.0% of ALH cases were pure, 40.5% were associated with pre-invasive DN and 14.5% with IDC. 31.6% of LCIS cases were pure, 39.6% were associated with pre-invasive DN and 28.8% with IDC.

Comparison of lobular and ductal neoplasia cases

			DN (Ductal Neoplasia)				
LN	No DN	FEA	ADH	DCIS	IDC	Total	
ALH	124 (14.81%)	7 (0.84%)	67 (8.00%)	38 (4.54%)	40 (4.78%)	276	LN+DN=46.7%
LCIS	79 (9.44%)	1 (0.12%)	43 (5.14%)	55 (6.57%)	72 (8.60%)	250	LN alone=53.3%
ILC	243 (29.03%)	0	9 (1.08%)	45 (5.37%)	14 (1.67%)	311	
							837

Conclusions: 1) LN is frequently associated with some form of DN and this association is not limited to FEA and tubular carcinoma. 2) The actual incidence of these associations may be higher than reported as FEA and ADH may go unnoticed in ILC cases. 3) Close association of ductal and lobular neoplasia supports that FEA is a pattern at the low end of the spectrum of low grade DCIS and ADH rather than a distinct entity and may explain the reported occurrence of tubular carcinoma in followup studies of LCIS.

237 DCIS in African-American (AA) vs. Caucasian-American (CA) Women: Analysis of Pathologic Features and Outcome

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Background: AA women are diagnosed with invasive breast carcinoma at younger age, and at more advanced stage than CA. Tumors in AA have a more aggressive phenotype; their mortality rate is significantly higher than that of CA, mainly among younger patients. There is little information in the literature however on the characteristics of DCIS in AA patients.

Design: We studied AA and CA patients diagnosed with DCIS between 1996 and 2000 at our institution, excluding patients with a previous diagnosis of invasive carcinoma or who recurred as invasive carcinoma within 6 months of diagnosis. Cases with microinvasion were included. We reviewed the H&E slides (to assess grade, presence of central necrosis) and pathology reports (to obtain the size) of the DCIS. Descriptive statistics, treatment, and outcome were obtained from the patients' clinical charts and the SEER database.

Results: We identified 251 AA (61%) and 164 CA (39%) patients with DCIS with a mean age at diagnosis of 60 y and 56 y respectively. 40% of AA patients were diagnosed when older than 64y of age compared to 20% of the CA (p =0.0004). Except for T size which was larger in AA (1.9 cm vs.1.4 cm; p=0.002), there was no difference in the pathological features of DCIS between the two groups (grade, central necrosis, ER/PR). CA patients underwent mastectomy as a final procedure more often than AA (36% vs. 26%; p= 0.03). Other treatment modalities (hormone and radiation therapy) were similar between the two groups. Recurrence as DCIS (no invasion) occurred in 5% of AA and 4% of CA (38m f/u, p=NS) and did not correlate with the various pathological parameters. Recurrence as invasive carcinoma occurred in 6% of AA and 3% of CA (45m f/u, p=NS). There was a significant correlation between recurrence as invasive carcinoma and T size, presence of microinvasion (in AA and CA), and central necrosis (only in AA). Death due to disease was higher in the AA population (4% vs. 0.6% with a mean f/up of 84 m, p=0.03) and occurred in patients who recurred with invasive carcinoma. The 10-year DFS rate for AA and CA were 87% and 95% respectively (p=0.001).

Conclusions: In our patient population, DCIS is diagnosed at a later age in AA women. Except for larger size, DCIS does not have more aggressive histology in AA. Recurrence as invasive carcinoma correlated with T size, microinvasion and central necrosis. Although recurrence was similar in both groups, AA women with DCIS appear to have a worse survival than CA.

238 The Use of $p16^{INK4a}$, but Not $p14^{ARF}$ or $p15^{INK4b}$ as a Potential Marker for Breast and Ovarian Neoplasms

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Background: The cell cycle kinase inhibitors $p14^{ARF}$, $p15^{INK4b}$, and $p16^{INK4a}$ undergo dysregulation of expression in multiple human cancers. The coordinated expression of these proteins in breast and ovarian cancer has not been described. In this study, we analyzed the expression of $p14^{ARF}$, $p15^{INK4b}$, and $p16^{INK4a}$ using the immunoperoxidase method, utilizing breast and ovarian cancer tissue microarrays.

Design: Tissue microarray slides containing 60 invasive ductal breast carcinomas and 60 high grade serous ovarian carcinomas were studied using antibodies to $p14^{ARF}$, $p15^{INK4b}$, and $p16^{INK4a}$. Staining intensity of these antibodies was measured using image analysis, and was then compared to the staining intensity of ten samples of non-neoplastic breast and ovarian tissue.

Results: Expression of $p16^{INK4a}$ was positive in 80% of the breast and ovarian carcinomas, while non-neoplastic breast and ovarian tissue was consistently negative. Expression of $p14^{ARF}$ and $p15^{INK4b}$ was observed in non-neoplastic breast and ovarian epithelial cells, with comparatively decreased expression in tumors (Kruskall-Wallis $p<0.001$). All three markers showed a significant direct correlation with each other (Spearman rank correlation $p<0.001$), with the exception of $p16^{INK4a}$ with $p15^{INK4b}$.

Conclusions: Of the cell cycle kinase inhibitors $p14^{ARF}$, $p15^{INK4b}$, and $p16^{INK4a}$, only $p16^{INK4a}$ expression is identified in the majority of breast and ovarian carcinomas. Expression of $p16^{INK4a}$ is not seen in non-neoplastic breast and ovarian tissue. Therefore, expression of $p16^{INK4a}$ may aid in the diagnosis of breast and ovarian carcinomas in difficult cases, particularly when the available tissue is scanty.

239 Application of the ASCO/CAP Guidelines for HER2 Testing to mRNA Expression Values from Microarrays

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Background: We previously identified a potential cutoff for HER2 mRNA expression values from Affymetrix U133 microarrays (Lancet Oncol, 2007; 8:203-11). Our goal was to evaluate this method in the context of the recent ASCO/CAP guidelines for HER2 testing.

Design: We measured HER2 mRNA expression levels from Affymetrix U133 microarrays obtained from clinical needle biopsies of 393 patients with newly diagnosed breast cancer. We compared these data with HER2 status that was defined by immunohistochemistry (IHC) in 252 samples, fluorescence in-situ hybridization (FISH) in 329 samples (Vysis, Des Plaines, IL) and combined IHC and FISH in 393 samples. HER2 gene expression (mRNA), expressed as a continuous variable, was compared to HER2 status using receiver operating characteristic (ROC) analysis. An equivocal range for mRNA gene expression was 10% above and below our published cutoff value. Agreement represents the comparison of negative or positive status, excluding equivocal cases.

Results: HER2 status was diagnosed as negative, equivocal or positive with the following frequencies: mRNA (72%, 5%, 24%), IHC (60%, 19%, 22%), FISH (70%, 3%, 24%), and combined IHC and FISH (73%, 2%, 24%). The accuracy of HER2 mRNA measurement is summarized in the table below.

Comparison of HER2 mRNA status with IHC and FISH status

HER2 mRNA (n=393)	IHC (n=252)	FISH (n=329)	Conbined IHC and FISH (n=393)
Area under ROC	0.93	0.96	0.95
Agreement	94%	93%	94%

Microarray-based mRNA expression values would convert HER2 status from negative to positive in 5 of 150 (3%) patients evaluated by IHC, 13 of 229 (6%) evaluated by FISH, and 13 of 288 (5%) evaluated by combined IHC and FISH. Of the nine cases that were equivocal after combined IHC and FISH analysis, seven were negative and two were positive by mRNA expression profiling.

Conclusions: Microarray-based mRNA expression values accurately represent HER2 status defined by IHC, FISH, or combined IHC and FISH. The area under the ROC curve provides the most reliable assessment of this continuous variable, and is in keeping with the accuracy level suggested by ASCO/CAP guidelines. Selection of an optimal threshold and equivocal range deserves further attention before this method could be used as a complement to standard HER2 testing.

240 Histologic Predictors of False Negative Sentinel Lymph Nodes Following Preoperative Chemotherapy

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Background: Sentinel lymph node (SLN) biopsy following preoperative chemotherapy has been controversial. A recent meta-analysis of twenty-one studies, including 1273 patients who underwent SLN biopsy and axillary dissection following preoperative chemotherapy, concluded that SLN biopsy following preoperative chemotherapy is reliable. The National Surgical Adjuvant Breast and Bowel Project B-27 (NSABP B-27) recently reported a successful identification rate and false negative rate of 84.8% and 10.7%, respectively. The purpose of this study was to determine whether histologic findings in negative SLNs could help to predict a false negative SLN biopsy.

Design: One-hundred thirty-three patients with operable breast cancer and node-positive disease confirmed by ultrasound-guided FNA underwent SLN biopsy and axillary dissection following preoperative chemotherapy between 1994 and 2006. Available H&E sections of the false negative SLNs were reviewed, and any findings suggesting treatment effect in an area where metastatic tumor might have been present prior to chemotherapy were recorded. A false negative SLN was defined as a SLN with no evidence of metastatic carcinoma in a patient with metastatic carcinoma identified in at least one of the remaining nonsentinel lymph nodes.

Results: Eighty patients in the study had at least one positive SLN (60%), and 53 patients had negative SLNs (40%). The false negative rate was 15%. Fifty negative SLNs had histologic sections available for review. Twenty-four cases had focal areas of fibrosis, with or without hemosiderin deposition or calcification, focal areas with a foamy histiocytic infiltrate, or granulomas. These histologic findings occurred in 22/36 patients (61%) with true negative SLNs and in only 2/13 patients (15%) with false negative SLNs (Pearson's chi square analysis, P=0.005).

Conclusions: In node-positive patients who undergo preoperative chemotherapy and subsequent SLN biopsy, a negative SLN with no histologic evidence of treatment effect (such as fibrosis or a foamy histiocytic infiltrate) appears to have a higher likelihood of being a false negative SLN.

241 Papillary Lesions Diagnosed by Core Needle Biopsy of the Breast: Diagnostic Pitfalls and Management Update

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Background: Papillary breast lesions may present a challenge when diagnosed on core needle biopsy (CNB). There have been contradictory reports on incidence of malignancy at excision, and there are no uniform management recommendations. We have retrospectively reviewed CNB and excisions with mammographic and clinical data on all papillary lesions diagnosed at our institution by CNB.

Design: From 1995 to 2007, 209 lesions in 193 women (age 30-90, median 54.5) were diagnosed as papillary. 139 (67%) lesions presented with a mass, 17 (8%) with nipple discharge; these were biopsied under ultrasound guidance with 14g needle. In 53 (25%) lesions biopsy was performed for microcalcifications under stereotactic guidance using 11g vacuum-assisted probe. Of 209 papillary lesions, 152 (73%) were diagnosed as benign, 37 (17%) atypical, and 20 (10%) malignant (papillary DCIS). Excision was performed in 112.

Results: Malignancy was found on excision in 12 of 70 (17%) excised benign papillomas (including one invasive carcinoma-ICA), 12 of 27 (44%) atypical papillomas (including one ICA), and all 14 malignant papillary lesions among which 5 cases were upgraded to ICA including one invasive solid papillary CA, and one medullary CA with cystic change. Malignancy at surgery was seen more often in multiple peripheral than single subareolar papillomas (24% vs.4%, $p<.05$). Small incidental papillomas confined within the tissue core were never associated with upgrade to cancer. There was a trend for higher incidence of malignancy in benign papillomas biopsied for calcifications ($p=.07$). Papillomas with florid hyperplasia had higher likelihood of atypia but not malignancy at excision. 6 of 8 atypical papillomas downgraded to benign on excision showed only florid hyperplasia. 3 of 4 atypical papillomas with apocrine metaplasia showed cancer at surgery. In benign papillomas, morphologic features such as sclerosis and apocrine metaplasia, or other factors: family and individual history, gauge and number of cores did not predict outcome. Some benign entities such as adenomyoepithelioma and ductal adenoma were diagnosed as papillomas on CNB.

Conclusions: Excision should be recommended for most benign papillomas excluding small incidental lesions. Peripheral papillomas are associated with higher rate of malignant follow-up, whereas single subareolar lesions may be managed conservatively. We describe potential pitfalls and unusual lesions in the diagnosis of both benign and malignant papillary lesions on CNB.

242 Is There Molecular Evidence for Progression of Microglandular Adenosis to Carcinoma: Results of Comparative Genomic Hybridization (CGH) on Microdissected Lesions

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Background: Microglandular adenosis (MGA) is an uncommon, benign entity arising in the breast. Although most cases run an indolent course, histologic progression into atypical MGA, carcinoma in-situ (CIS), and invasive carcinoma (InvCa) occurs in rare instances. The purpose of this study is to characterize molecular evidence of tumor progression in MGA.

Design: Archival paraffin blocks from 16 female patients (pts) with a fundamental diagnosis of MGA were retrieved from our breast consultation files. Unstained sections were used for immunohistochemistry, special stains, and CGH. Areas representative of different stages of tumor progression (MGA, atypical MGA, CIS, InvCa) were separately microdissected by laser capture prior to CGH analysis.

Results: Of the 16 pts (age range: 42-86 years; mean 64; median 61) four had MGA only and six patients had InvCa (five with concurrent CIS and one with atypical MGA). Four pts had atypical MGA and CIS. The remaining two pts had MGA with either CIS or atypical MGA, respectively. Preliminary CGH data of four pts demonstrate concordance in the molecular profiles between different lesions from the same pt suggesting the lesions are clonally related. For example, in one pt both atypical MGA and CIS harbored loss of chromosome 5, 8p and 17 and gain of 8q and a focal gain on 17q. In a second case, atypical MGA had gain of chromosome 10 and loss of 16q and 17p whereas the concurrent CIS had loss of 6q, 16q and 21. CGH analysis is nearing completion for the remaining 12 cases.

Conclusions: The preliminary molecular data are consistent with MGA being, in some instances, a non-obligate precursor lesion of invasive carcinoma. The genetic changes are related to low grade tumors (16q loss) and high grade tumors (8q and 17q gain) suggesting a heterogeneous spectrum of MGA lesions. The challenge to improve pt management is to identify molecular markers of MGA which are more likely to progress to invasive carcinoma.

243 Performance Comparison of a Digital Pathology Solution for IHC to Conventional Manual Microscopy: A Feasibility Study Using 20 HER2 Stained Breast Specimens

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Background: Immunohistochemistry (IHC) Image Analysis (IA) systems today are stand-alone systems that are used as an adjunct to manual microscopy and therefore require extra work by labs and pathologists. Digital pathology (DP) integrates IA seamlessly into the digital workflow, allowing running IA with the push of a button while reading a digital slide on a monitor. As a DP IHC solution is going beyond the IA, the performance of the system for reading digital IHC slides on a monitor becomes equality important to the IA.

Design: A feasibility study was conducted at Pathology Inc. to compare the performance of Aperio's DP IHC solution to manual microscopy evaluating HER2 IA and reading digital HER2 slides on a monitor. A set of 20 formalin-fixed, paraffin-embedded breast tissue specimens stained using Dako's HerceptTestTM with equal HER2 score distribution were assayed. 3 pathologists performed a blinded read of the glass slides using a microscope. The slides were scanned and after a wash-out period of over one week and randomization the same 3 pathologists performed another blinded read of the same slides, but this time of the digital slides on a monitor. At the same time, each pathologist also outlined a representative set of tumor regions to be analyzed by the IA algorithm. The algorithm itself was run in batch mode blinded from the pathologists to avoid influencing the pathologists in their choice of the tumor regions. The algorithm was used with the provided default parameter set for Dako's HerceptTest. The algorithm reported the HER2 score for each of the 3 pathologists for each of the slides.

Results: Each of the methods: manual microscopy (MM), reading a digital IHC slide on a monitor (DR) and image analysis (IA) were evaluated separately and comparatively between methods using Percent Agreement (PA) of the clinical relevant dichotomous outcome of negative HER2 scores (0, 1+) vs. positive HER2 scores (2+, 3+). The study showed comparable PA values for DR and MM (80.0%-90.0% MM, 75.0%-90.0% DR, 75.0%-90.0% MM vs. DR), and also for IA and MM (80.0%-90.0% MM, 80.0%-85.0% IA, 80.0% - 90.0% IA vs. MM).

Conclusions: This feasibility study supports the substantial equivalence of Aperio's DP IHC solution to manual microscopy and it is expected that a larger multi-site study will provide the required performance validation data for a regulatory clearance.

244 C1772T (P582S) or G1790A (A588T) Polymorphisms of *HIF-1*α Gene and HIF-1α Protein Expression in Breast Cancer Patients

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Background: Hypoxia inducible factor 1 (HIF-1) is an important genetic component involved in the cellular response to hypoxia. HIF-1 is also linked to the regulation of tumor development and growth. In previous studies, P582S or A588T polymorphisms of the human HIF-1α gene have been identified in renal cell carcinoma, head and neck, and esophageal squamous cell carcinomas as well as colorectal and prostate cancers.

Design: We investigated whether polymorphisms of the HIF-1α gene may account for the expression patterns of HIF-1α protein in 90 breast cancer patients by using PCR, sequencing and immunohistochemistry, performed on the tissue microarray. We also examined the role of HIF-1α gene polymorphism and protein expression in the prediction of biological behavior.

Results: The frequency of the T allele for P582S in 90 breast cancer patients and 102 healthy controls was 5.6% and 4.4%, whereas, the frequency of the A allele for A588T was 1.7% and 4.4% respectively. There was no difference in genotype distribution for the polymorphisms between breast cancer patients and healthy controls. However, a positive association of the P582S polymorphism and increased HIF-1α protein expression, but not for A588T, was found in breast cancer patients ($p<0.04$). Increased HIF-1α protein expression was found in cases of breast cancer with lymph node metastasis ($p<0.04$), high histologic grade ($P<0.001$) and increased Ki-67 index ($p<0.03$).

Conclusions: These results suggest the potential use of P582S polymorphism and HIF-1α protein expression analysis in providing a new prognostic factor for unfavorable disease outcomes and may help for clinical decision-making in the treatment of breast cancer patients.

245 Lower Levels of CD8+ Intratumoral T Cells Is Associated with Poor Prognosis in Breast Carcinoma of Premenopausal African American Women

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Background: Premenopausal African American (AA) women have an increased incidence of high grade hormone receptor negative breast carcinomas, for which the pathogenesis remains unknown. These tumors are frequently associated with abundant levels of tumor infiltrating lymphocytes which however appear ineffective in abrogating the tumor. We hypothesized that this failure of the immune system to elicit an anti-tumor immune response may be attributed to CD4+Foxp3+ regulatory T cells which inhibit CD8+ anti-tumor T cell responses.

Design: We evaluated 12 high grade infiltrating ductal breast carcinoma cases negative for ER, PR and Her 2/neu. Five of the cases were premenopausal AA women. We studied the extent of lymphocytic infiltration on H&E stained sections and then immunostained for CD4, CD8, Foxp3 and perforin. T cells which were inside the tumor cell nests were categorized as intratumoral lymphocytes and those which were peripheral to the tumor nests were categorized as intertumoral lymphocytes. T cells were counted manually and on scanned digital images using an automated cell imaging system (ACIS, Clarient).

Results: In both the premenopausal AA women (group a) and in non-premenopausal AA women (group b), the intertumoral CD8+/CD4+ ratios were 2:1 and the % of CD4+ T cells which were Foxp3+ in group a=15.2, SD=8.0 and in group b=18.4, SD=8.0. However, the average intratumoral CD8+/CD4+ ratio in group b=8:1 but only 3:1 in group a. Thus, in comparison to the intertumoral CD8+/CD4+ ratio, the intratumoral CD8+/CD4+ ratio increased more than 4 fold in 5/7 patients in group b but none (0/5) of the premenopausal AA women had such an increase. This brisk tumor infiltrating lymphocytic population could be seen on the H&E stained sections in group b but was rarely seen in group a. The functional behavior of the CD8+ T cells was verified by perforin immunostain.

Conclusions: We observed a decreased ratio of intratumoral CD8/Treg cells in premenopausal AA women vs. non-premenopausal AA women, which could not be attributed to different Foxp3+ regulatory T cells. This decrease in anti-tumor T cells may contribute to the poor prognosis of young AA women. In all cases of infiltrating breast carcinomas, CD4+Foxp3+ cells were a significant percentage of the intertumoral

lymphocytes and may contribute to the inability of the immune system to reject the tumor. In these ER, PR, Her 2/neu negative breast carcinomas, CD4+ Foxp3+ regulatory T cells are a potential therapeutic target.

246 Standardizing Breast Sentinel Lymph Node Processing: The Role of a Real Time Reverse Transcriptase – Polymerase Chain Reaction Assay

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Background: Despite current College of American Pathologists' guidelines, SLN processing remains variable in terms of performing multiple tissue levels, immunohistochemical (IHC) or PCR-based assays. A rapid and reliable molecular pathology assay, as an adjunct to routine SLN processing, could minimize or even standardize the histologic evaluation needed for accurate and clinically significant diagnosis. We compared the Veridex GeneSearch™ Breast Lymph Node (BLN) Assay (Veridex, LLC; Warren, NJ), a real time reverse transcriptase-polymerase chain reaction assay that is designed to detect clinically significant metastases (≥0.2 mm), with our standard lymph node processing.

Design: Fifty-seven patients were assessed for the assay's performance in SLN tissue that would normally be deep within the tissue block and not routinely evaluated histologically in order to preserve our routine sampling of the SLN; this differs from the manufacturer's recommended sampling of half the node. In our protocol, two 1mm slices from the outer node portions are submitted fresh for RNA extraction. The SLN is then bisected longitudinally and embedded with both halves down. Twenty-one slides are cut from each block; slides 1, 7, 8, 14, 15 and 21 are routinely evaluated by H&E and IHC is performed on one set of the unstained slides. The BLN assay evaluates RNA expression data for three target genes (mammaglobin, cytokeratin 19, and internal control porphobilinogen deaminase) in the fresh tissue. The gene expression results are then applied against predetermined criteria to provide a qualitative (positive/negative) result. These results were not used for patient management.

Results: From the 57 patients, the assay assessed 42 as true negative, 7 as true positive (metastases 7–20mm), 1 as false negative (metastasis 7 mm), 3 as false positive, and 4 as invalid, when compared to histologic diagnoses. Assay sensitivity was 87.5% (7/8), specificity 93.3% (42/45), negative predictive value 97.7% (42/43), and positive predictive value 70.0% (7/10).

Conclusions: The assay allows for efficient evaluation of tissue normally not tested. Sensitivity of the assay using only the outer SLN tissue is high (87.5%) and is identical to that validated in the large registration study in which half of the node was assessed (sensitivity = 87.6%). This assay as an adjunct to traditional histologic evaluation could minimize and ultimately standardize the number of histologic sections needed for thorough SLN evaluation.

247 Triple Negative Breast Cancer: Molecular Profiling and Prognostic Impact in Adjuvant Anthracycline-Treated Patients

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Background: Oestrogen receptor (ER), progesterone receptor (PR) and HER2 negative (i.e. triple negative (TN) breast cancers are an aggressive subtype of breast cancers. TN phenotype has been used as a surrogate for basal-like (BL) phenotype, however it is unclear whether the overlap between these two groups is sufficiently accurate. Furthermore, the prognosis of patients with TN cancers treated with adjuvant anthracycline-based chemotherapy has not been studied. Here we compared the distribution of basal markers and biomarkers, the prevalence of amplification of oncogenes, and the outcome of TN vs non-TN cancers.

Design: We examined the prognostic impact of the TN and BL phenotype in 245 breast cancer patients uniformly treated with therapeutic surgery followed by standard dose adjuvant anthracycline-based chemotherapy with regards to local relapse-free (LRFS), metastasis free (MFS), and breast cancer specific (BCSS) survival. We performed a comprehensive comparative analysis of the clinicopathological characteristics, expression of basal markers (cytokeratins (Cks) 5/6, 14, 17, EGFR, and caveolin 1 and 2), Ki67, p53 and topoisomerase II alpha, and prevalence of *CCND1*, *MYC* and *TOP2A* gene amplification as defined by chromogenic *in situ* hybridisation in TN and non-TN breast tumours.

Results: TN cancers were significantly associated with the expression of basal markers including Cks 5/6, 14, 17, EGFR and caveolins 1 and 2 (all $p<0.0001$). However, we observed that 6/31 (19.4%) TN tumours were negative for basal markers, whilst expression of these markers was seen in 15/207 (7.3%) non-TN tumours. TN phenotype was significantly associated with p53 nuclear accumulation and high proliferation rates as defined by MIB-1 and topoisomerase II alpha (all, $p<0.01$) expression. No TN cancer harboured amplification of *CCND1* or *TOP2A*. In univariate analysis, TN and BL phenotype were significantly associated with shorter MFS (both, $p<0.01$) and BCSS (both, $p<0.005$) but not LRFS.

Conclusions: Despite treatment with standard dose anthracycline-based chemotherapy, the clinical outcome of TN and BL cancers remains poor. Alternative chemotherapeutic regimens and/or novel therapeutic approaches are warranted. Although a significant phenotypic overlap exists between TN and basal-like tumours, the TN phenotype should not be used as an ideal surrogate marker for basal-like breast cancers.

248 Invasive Solid Papillary Carcinoma of the Breast: A Clinicopathologic Analysis of 52 Cases

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Background: Breast carcinomas with neuroendocrine differentiation (NE) are uncommon tumors. They comprise a spectrum of tumors with different histolomorphology and clinical behavior. Solid papillary carcinoma (SPC) is mostly considered as an in situ carcinoma with NE differentiation. Large series of invasive SPC have not been reported.

Design: We define the invasive SPC as following: A) invasive carcinoma comprises majority of the tumor, the in situ component comprises 25% or less of the entire tumor B) the invasive component has solid papillary configuration C) 50% or more of the invasive tumor cells show NE differentiation (positive for synaptophysin and/or chromogranin). Cases of other histologic morphology with NE differentiation are excluded. 52 cases of invasive SPC from 51 patients (49 females and 2 males, one female with bilateral invasive SPC) with mean follow up of 14 months (1 to 189 month) were identified in our file. Clinicopathologic features were evaluated.

Results: Patient age range from 28 to 82 y/o with a mean of 59 y/o. Regardless of stage, significant amount of the tumors (31%) show lymphovascular invasion and high rate of axillary lymph node metastasis: 8 /24(33%) T1 tumor, 10/24 (42%) T2 tumor and 3/3 (100%) T3 tumor. Significant rate of local recurrence (12%) and distal recurrence (22%) are also noted. Kaplan-Meier analysis reveals 10% risk for local recurrence at 2 years, 25% at 5 years; 18% risk for distal recurrence at 2 years and 30% at 5 years. The prognosis of invasive SPC is excellent despite the high incidence of lymphovascular invasion, high rate of axillary lymph node metastasis, local and distal recurrence. None of the patients die of disease, including twelve patients with longer than 5 year follow-up.

Conclusions: Invasive SPC is a variant of invasive carcinoma with NE differentiation. The age of onset seems similar to other invasive breast carcinoma, but significantly younger than that of in situ SPC, or in situ SPC with associated invasive carcinoma. In our study, the in situ carcinoma associated with invasive SPC are predominantly other types instead of solid papillary.Seventeen out of 52 cases show no in situ carcinoma. Based on these findings, we think the invasive SPC is unlikely arising from in situ variant of SPC. It has its own unique clinicopathologic features distinguished from other types of breast carcinomas.

249 Bone Metastasis Is Strongly Associated ER Positive/PR Negative Breast Carcinomas

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Background: Bone is one of the most common sites for distant metastasis of breast cancer. In this study, our objective is to identify molecular markers and moelcular classifications that may predict which patients are at risk of developing bone metastasis.

Design: Immunohistochemical analysis was performed on representative sections of 21 breast carcinomas with bone metastasis and 94 cases without bone metastasis to antiobodies against ER, PR, AR, HER2, EGFR, CK5/6, CK14, CK17, CK8 and CK18. The expression rates of receptors and subtype distributions of 3 molecular classifications were compared between these two groups. All 3 molecular classification divide breast carcinomas into basal and non-basal subtypes with the basal subtypes defined as follows: CK5/6, CK14 and/or CK17 positive for Cytokeratin (CK) classification; ER, PR and HER2 negative for Triple Negative (TN) classification; and CK5/6, CK14, CK17, and/or EGFR positive and ER, PR, HER2 negative for CK/TN classification.

Results: Among the 21 patients with bone metastasis, the mean age was 50 for primary cancers and 57 for bone metastasis with a range between breast primary and bone metastasis of 0-25 years and a mean of 9 years. Seventeen cases were ductal carcinoma and 4 were lobular carcinoma. Fourteen cases were grade 1-2 tumors and 7 were grade 3. The comparison between carcinomas with or without bone metastasis is shown in Table 1.

Comparison beween breast carcinomas with or without bone metastasis

	Invasive carcinoma without bone metastasis (94 cases)	invasive carcinoma with bone metastasis (21 cases)	p-value
receptor expression (%)			
ER+	55 (59%)	18 (85%)	0.0235
PR+	49 (52%)	10 (48%)	0.8107
ER+/PR-	6 (6%)	7 (33%)	0.0022
AR+	70 (74%)	20 (95%)	0.0412
HER2+	18 (19%)	5 (23%)	0.7629
EGFR+	6 (6%)	1 (5%)	1.0000
Molecular classification			
Cytokeratib (CK)			1.0000
Non-basal	76 (81%)	17 (81%)	
Basal	18 (19%)	4 (19%)	
Triple Negative (TN)			0.2361
Non-basal	72 (77%)	19 (81%)	
Basal	18 (19%)	4 (19%)	
CK/TN			0.4603
Non-basal	81 (86%)	20 (96%)	
Basal	13 (14%)	1 (4%)	

Conclusions: 1. Bone metastasis is strongly associated ER positive/PR negative tumors. 2. Expression rates of PR, HER2, EGFR and 3 molecular classifications may not be useful in predicting bone metastasis.

250 Estrogen Receptor Positivity in Normal Breast Epithelium from Women with Breast Cancer Correlates with Tumor Hormone Receptor

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Background: Increased estrogen responsiveness of breast may contribute to development of breast cancer (BrCa). Estrogen receptor (ER) expression in normal breast (NBR) epithelium of women with BrCa has been reported to be higher than in non-cancer controls. However in women with BrCa correlation between ER expression in NBR epithelium and tumor characteristics has not been studied. The aim of this study is to evaluate ER expression in NBR tissue in women with BrCa and to correlate it with hormone receptor status of the tumor.

Design: 1 mm tissue micro-arrays (TMA) of NBR tissues were constructed from 84 women with breast cancer. ER expression was evaluated using IHC staining. Percentage of ER positive cells in normal acini (A) and ducts (D) were separately recorded. All staining were considered positive without grading the intensity. Staining in normal epithelium was evaluable for one TMA core for each of 48 subjects and from two TMA cores for each of 36 subjects. We summarized % of normal cells stained according to clinico-pathologic features of the tumor and conducted nonparametric tests differences.

Results: The mean percent of ER+ acini was 46.5 (sd 20.8; median 46, range 1 - 100), and for duct cells the mean was 53.3 (sd 27.4; median 52, range 8-98). The mean and median ER+ cells did not vary by age or menopausal status. However, the standard deviation and range were larger among pre-menopausal women (mean acinar cells ER+ 45.1, sd 24.7) than post-menopausal women (mean 49.9, sd 16.2) consistent with cyclic changes. ER expression in NBR was not associated with ER expression in tumor ($p = 0.87$). However, NBR acinar (A) cells from women with PR positive tumors (mean 51.7, sd 21.2; median 54, range 5.5-100) had significantly higher percent ER positivity as compared to women with PR negative tumors (mean 43.5, sd 19.9; median 44, range 1-91), $p=0.04$; the association with PR in tumor was more pronounced among postmenopausal women ($p=0.007$).

Conclusions: Percentage of ER+ cells in NBR from women with BrCa was higher than the 10% reported in women without BrCa. ER positivity rate was high in women with BrCa and was similar in pre- and postmenopausal groups. ER positivity in NBR of these women is significantly associated with tumor progesterone receptor.

251 Expression of Fatty Acid Synthase (FAS) in Columnar Cell Lesions of the Breast

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Background: Columnar cell lesions (CCLs) encompass a spectrum of potentially premalignant histologic changes characterized by the presence of columnar-shaped cells in enlarged terminal duct lobular units (TDLUs). The risk associated with CCLs has not yet been defined. FAS expression has recently been proposed as a marker of increased risk in pre-invasive breast cancer. We set out to delineate the incidence of CCLs, the association with invasive breast cancer, and the relevance of FAS expression in CCLs.

Design: We retrospectively reviewed H&E slides from 430 patients who received surgery for invasive breast cancer between 1984 and 2002. Columnar cell lesions were identified and classified as columnar cell change (CCC) and columnar cell hyperplasia (CCH), with or without atypia (using previously established histologic criteria). The expression of FAS protein was determined by immunohistochemistry in a subset of CCLs selected to represent these categories.

Results: Out of 436 samples, CCLs were identified in 189 cases (44%), including 43 CCCs without atypia, 23 CCCs with atypia, 56 CCHs without atypia, and 67 CCHs with atypia. These 189 cases included invasive ductal carcinomas no special type (135/328, 41.1%), invasive lobular carcinomas (40/71, 56.3%), tubular carcinomas (4/6, 66.7%), medullary carcinomas (2/8, 25%), cribriform carcinoma (1/3, 33.3%), and colloid carcinomas (5/8, 62.5%). Besides invasive tumors, CCLs were also associated with apocrine metaplasia (69/189, 36.5%), atypical ductal hyperplasia (6/189, 3.2%), ductal carcinoma in situ (125/189, 66.1%), and lobular carcinoma in situ (48/189, 25.4%). FAS was expressed in 25/39 (64.1%) CCLs, with higher immunoreactivity in atypical columnar cells. Superimposed apocrine metaplasia and in situ or invasive carcinomas were also positive, whereas normal TDLUs showed weak focal staining.

Conclusions: CCLs are common lesions associated more frequently with some subtypes of invasive breast tumors. FAS expression in CCLs is similar to other benign proliferative breast lesions. While not specific for CCLs, further study may be helpful in highlighting differences between subcategories of CCLs and may provide insight into the biology and natural history of these lesions.

252 Improving Interobserver Reproducibility of Nodal Stage Classification through Standardized Histologic Criteria and Image-Based Training

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Background: Reliable pathologic stage classification of axillary lymph nodes is an important determinant of prognosis and therapeutic decision-making for patients with invasive breast cancer. Pathologists' distinction between micrometastasis (pN1mi) and isolated tumor cells [ITC; pN0(i+)] is variable using the American Joint Committee on Cancer 6th edition staging manual. We sought to determine if a set of clearly defined histologic criteria could lead to reproducible nodal classification by an international group of pathologists.

Design: Digital images of sentinel lymph node biopsies from 56 patients with small volume nodal metastases were examined by six experienced breast pathologists (MDs), first as a Pre-Test, and again as a Post-Test after studying a Training Program that outlined and illustrated the classification criteria. Multi-rater agreement and kappa statistics compared results from individual MDs and the reference MD.

Results: Multi-rater analysis of the six MDs improved from 71.5% (Pre-Test kappa 0.487;ASE 0.039) to 95.7% (Post-Test kappa 0.915;ASE 0.037). Compared to the reference MD, agreement improved from 76.2% (Pre-Test kappa 0.575, CI 0.25) to 97.3% (Post-Test kappa 0.947, CI 0.049). Agreement on lobular carcinoma metastasis classification improved from 55% (23/42; Pre-Test) to 100% (42/42; Post-Test) ($p<0.001$), and agreement on ITC classification in nodal parenchyma improved from 67.6%(69/102; Pre-Test) to 98.0%(100/102; Post-Test)($p<0.001$). The few persistent discordances on Post-Test were with tumor cell clusters at or near the 0.20mm threshold.

Conclusions: Application of current definitions for classification of small volume nodal metastases are inconsistent leading to variable classification of ITCs and micrometastases. Reproducibility of pathologic nodal stage classification is achievable with well defined histologic criteria and an image-based training set to clarify the AJCC 6th edition criteria.

253 Expression of EGFR, IL-1, Amphiregulin, and NF*k*B in Triple Negative Breast Cancer, and Their Correlation with Histologic Findings

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Background: It has been shown that in cells overexpressing EGFR, when EGFR is activated via autocrine stimulation by the growth factor Amphiregulin (AR), downstream signaling is dramatically altered resulting in transcriptional upregulation of IL-1alpha and subsequent activation of the transcription factor NF-*k*b. This is presumed to be the primary pathway stimulating growth and metastases in these cells, and might explain the less than favorable results of EGFR inhibition in EGFR positive patients. The aim of our study is to describe the expression of these markers in triple negative breast carcinomas and correlation with different pathological parameters.

Design: We retrospectively selected 52 patients with triple negative breast carcinomas undergoing mastectomy or lumpectomy between 2004 and 2007. Tissue microarray (TMA) was constructed using two cores from tumor area and one core from uninvolved breast tissue from formalin-fixed paraffin embedded tissue. Immunohistochemical stains for EGFR, IL1, AR, NF*k*B were performed. The cases were scored as positive and negative (with at least 10% of the tumor cells staining considered positive for EGFR, AR, and IL-1 and 25% of tumor cells staining for NFkB). Patients' demographic information and histologic findings were reviewed.

Results: Mean age of patients was 53.7 years (SD ± 12.2). All tumors were high grade with a mean size of 3.2 cm (SD ± 3.42). 60% of cases were associated with high grade DCIS, 52% with angiolymphatic invasion, and 42% with lymph node metastasis. Sixty-seven percent of cases were EGFR positive, 79% were IL-1 positive, 61% AR and 56% NFkB positive. Eleven cases (22%) were positive for all four markers. There was a significant correlation between expression of AR and NFkB (p=0.05). There was no correlation however, between AR and EGFR, and IL1. Expression of AR was significantly higher in patients with angiolymphatic invasion ($p=0.01$) but not with lymph node metastases.

Conclusions: A high proportion of triple negative breast carcinomas express EGFR, AR, IL1, and NFK. Several studies describe a role for AR in aggressive forms of breast cancer; our study confirms these findings. We also show a relation between AR and NFkB. The latter has also been previously associated with aggressive forms of breast cancer and pancreas cancer. Once NF-κB has been activated and translocated to the nucleus, it activates a repertoire of genes associated with inflammatory processes, and processes associated with invasion and metastasis.

254 A Q-RT PCR Profile for the Cytological Diagnosis of Early Breast Lesions

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Background: Preoperative cytological or histological diagnosis of suspicious breast lesions is mandatory in order to avoid unnecessary surgical biopsies and plan the surgical technique. However, both techniques are associated with a 3-6% rate of false-negative results and a small rate of false-positives. We aimed at identifying biomarkers that might constitute a simple, quick, reproducible and reliable diagnostic tool using fine needle aspiration (FNA) specimens.

Design: We have previously compared Agilent 44K-gene expression profiles of 144 breast cancers to normal breast tissue. 666 genes were significantly deregulated in more than 130 tumors. Q-RT-PCR expression of the 22 top genes was evaluated in a first exploratory series of 10 malignant and 11 benign breast lesions. mRNA were extracted from FNA specimens using the Qiagen RNEasy Kit. Gene expressions were normalized on the average level of 18S, GAPDH, RPLPO. Genes significantly differentially expressed were evaluated in a second series of 114 FNA samples, prospectively collected in 105 consecutive pts within a 3-month period at our one-stop breast unit. A cross-validation method was used (leave one-out method). We used a forward stepwise approach in an unconditional logistic regression model based on a likelihood ratio test in order to find a subset of the candidate genes associated with diagnosis of malignancy. We used the c-index (area under the ROC-curve) to summarize the ability of a model to classify risk.

Results: 6 genes out of the 22 first tested were highly significantly differently expressed between malignant and benign lesions in the exploratory series. The extended series

comprised 39 benign and 75 malignant lesions. Median age of the patients was 55 years (range: 18-92) and median size of lesions was 20 mm (range 5-110). 57% were classified Birad 5. Four out of the 6 genes were highly predictive of diagnosis (all four with p<10E-7). During cross-validation, class prediction of individual genes varied between 78 and 90%. In the multivariate analysis, the best-fit model comprises two genes with p values of .001 and .003. The c-value of the two-gene model is 0.96 [0.92-1.00]. The genes encode a protein involved in chromosome segregation and in invasion.
Conclusions: We identified a limited gene profile able to precisely classify suspicious breast lesions as benign or malignant and we are now performing a validation study.

255 Co-Expression of Cytoplasmic E-Cadherin and C-erbb2: Implications for Breast Tumor Invasion

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Background: Our previous studies showed that cell clusters overlying focal myoepithelial cell layer disruptions (FMCLD) had significantly elevated cytoplasmic expression of c-erbB2 and E-cadherin (Man et al. Cancer Detect Prev, 29:323-331, 2005; Man et al. Cancer Therapy 4:193-204, 2006). Since these two molecules are closely associated with tumor invasiveness and aggressiveness, respectively, this study intended to assess if they would be co-localized within the same cells.
Design: Consecutive sections were prepared from 50 previously identified such cases. The potential co-localization of E-cadherin and c-erbB2 was evaluated with three technical approaches: [1] sets of two adjacent sections were immunostained for E-cadherin and c-erbB2, respectively; [2] a single section from each case was double immunostained for E-cadherin and c-erbB2 using two different chromogens; [3] immunoreactive cells and adjacent cells within the same duct were microdissected and assessed for the mRNA levels of these two molecules with Real-time RT PCR.
Results: Intense cytoplasmic expression of c-erbB2 and E-cadherin was seen in a vast majority of the cell clusters overlying FMCLD. About 90% of c-erbB2 positive cell clusters were immunoreactive to E-cadherin. Both molecules were present predominantly in the cytoplasm, but adjacent cells within the same duct were largely negative or with membranous expression of these molecules. Most cells with cytoplasmic expression of E-cadherin and c-erbB2 were elongated or in spindle shape, in contrast to the round or oval shape in adjacent cells within the same duct. E-cadherin and c-erbB2 positive cells in most large clusters (<50 cells/cluster) were arranged as "tongue"-like projections invading deep into the stroma or vascular structures, which had an elevated proliferation index and were often in direct physical continuity with invasive lesions. Real-time RT PCR showed that microdissected cell clusters overlying FMCLD had a significantly elevated levels of both E-cadherin and c-erbB2 mRNAs.
Conclusions: The co-localization of E-cadherin and c-erbB2 within the same cells suggests that these two molecules might be functionally correlated, and that the shift of the sub-cellular localization of these two molecules might contribute to cell motility and invasion (Supported by grants PC051308, DAMD17-01-1-0129, DAMD17-01-1-0130 from Congressionally Directed Medical Research Programs, and BCTR0706983 from the Susan Komen Breast Cancer Foundation to Dr. Yan-gao Man).

256 Co-Localization of Wilms' Tumor 1 with Maspin and p63 in Mammary Myoepithelial Cells

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Background: Our previous immunohistochemical studies in a small number of human breast tissues revealed that Wilms' tumor 1 (WT-1) protein was preferentially expressed in normal myoepithelial (ME) cells (Man and Sang, Exp Cell Res 301: 103-118, 2004). Since WT-1 has both tumor suppressing and oncogene properties through its interactions with different molecules, this study attempted to assess whether WT-1 would be co-localized with two well-defined tumor suppressors, maspin and p63, which are present exclusively or preferentially in ME cells.
Design: Consecutive sections were prepared from paraffin-embedded human breast tissues from 20 patients with pre-invasive lesions and 20 aged-matched normal females with no family history of breast cancer and with no morphological breast abnormalities. Sets of 3 immediate adjacent sections were immunostained with monoclonal antibodies against WT-1 (Cell Marque, Hot Springs, AR), maspin, and p63, respectively. From each case, 5-7 randomly selected duct or acinar clusters with satisfactory immunostaining for all three antibodies were photographed. The expressing frequency and the sub-cellular localization of these three molecules in enlarged prints were examined and statistically compared.
Results: In both pre-invasive breast lesions and normal breast tissues, distinct WT-1 immunoreactivities were exclusively or preferentially present in morphologically distinct ME cells, with occasional diffusible immunoreactivities in endothelial cells or the lumen of ducts and acini. Similar to maspin, WT-1 appeared to be expressed mainly in the cytoplasm of ME cells. The percentage of WT-1 immunoreactive cells in focally disrupted ME cell layers was significantly reduced, compared to that in their non-disrupted counterparts. About 90% of the WT-1 positive cells were strongly immunoreactive to both maspin and p63. The absolute number of WT-1 positive ME cells, however, was significantly fewer than those of p63 and maspin positive cells.
Conclusions: The co-localization of WT-1 with known tumor suppressors suggest that WT-1 may interact with maspin and p63, to either enhance or inhibit the paracrine inhibitory functions of tumor suppressors on tumor cell growth. These findings also suggest that WT-1 may be a useful ME cell phenotypic marker. (Supported by research grants PC051308, DAMD17-01-1-0129, DAMD17-01-1-0130 from Congressionally Directed Medical Research Programs, and BCTR0706983 from the Susan Komen Breast Cancer Foundation to Dr. Yan-gao Man).

257 Longitudinal Analysis of Benign Breast Disease in Women Developing Breast Cancer after a Benign Biopsy: A Mayo Breast Disease Cohort Study

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Background: The histological progression of benign ("fibrocystic") breast disease (BBD) is poorly understood, owing partly to lack of longitudinal studies that compare pathologic findings at different time points. Such data would be crucial in better defining the relationship between BDD and breast carcinoma.
Design: We compared BBD changes in archival H and E stained sections from initial benign biopsies and in benign adjoining area sections from subsequent cancer bearing lumpectomy/mastectomy (lx/mx) specimens. The study included 520 women who developed breast cancer from a total of 10,969 in the Mayo Benign Breast Disease cohort (biopsied 1967-1991, mean follow up 11.8 yrs). In both specimens, BBD was classified as nonproliferative (NP), proliferative (P) or atypical (AH), using standard microscopic criteria, by one pathologist who was blinded both to outcome and sample identity.
Results: At time of initial biopsy, the frequency of NP, P and AH changes was 49%, 42% and 8.6%, respectively. Frequencies of NP, P and AH in subsequent lx/mx specimens were 23%, 25% and 52%, respectively (p<.01). Only 30% of women with NP on initial biopsy were also classified as NP in later lx/mx samples; 21% had P BBD and 49% had AH in lx/mx samples. In contrast, 69% of women with AH in their initial biopsy also had AH in their lx/mx; 13% of these changed to NP and 17% to P BBD. Among women with P BBD on initial biopsy, 31% were also P in subsequent lx/mx whereas 17% changed to NP; however, 52% had AH in their lx/mx.
Conclusions: Proliferative BBD and AH are significantly more frequent in cancer bearing specimens, compared to initial biopsies, in women from this cohort. These findings support the hypothesis that in women with BBD, most subsequent breast cancers evolve either directly from, or in association with, a progressive sequence of proliferative changes and that AH is a common intermediate step in all BBD subsets.

258 Analysis of Staining Heterogeneity and Level of Amplification in Breast Carcinomas with "2+" HER-2 Immunoreactivity

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Background: The significance of equivocal/moderate ("2+") HER-2 staining, particularly when partial/heterogeneous, is poorly understood.
Design: We compared the presence and degree of HER-2 amplification to the extent of HER-2 immunostaining (Dako HercepTest) in 201 "2+" cases that were also analyzed by FISH. Both tests were performed on the same block at a single lab to confirm eligibility for randomization into NCCTG 9831, a trial of Herceptin efficacy in locally advanced breast cancer. HER-2 staining was semiquantifed as <25%, 25-49%, 50-74% and >75% tumor cells with moderate, circumferential plasma membrane immunoreactivity. Amplification was defined as "marginal" when the HER-2 gene copy to chromosome 17 signal ratio was 2.0-4.0, "low level" when 4.1-6.0 and "high level" when >6.0.
Results: HER-2 amplification was present in 130/201 (65%) cases although it was "marginal" in 48% (62/130). Most tumors (55%, 110/201) exhibited HER-2 staining in less than 50% of tumor cells and only 8% had staining in ≥75% of cells. Likelihood of HER-2 amplification correlated significantly with extent of staining (p=<0.001; overall chi-square test): "high level" amplification was present in 11/16 (69%) of cases with ≥75% staining vs only 3/48 (6%) with <25%.

% of Tumor Cells with "2+" Stain

Signal Ratio	<25%	25-49%	50-74%	>75%
0 - 1.99	20	21	26	4
2.00 - 4.00	17	25	19	1
4.01 - 6.00	8	7	7	0
>6.0	3	9	23	11
TOTAL	48	62	75	16

Conclusions: HER-2 staining of "2+" cases is usually heterogeneous, adding to the difficulty of stain interpretation in this subset of breast cancers. The degree of HER-2 amplification in "2+" tumors is most often "marginal" or "low level". Although staining extent correlated with presence of gene amplification, "high level" amplification can sometimes occur in tumors with focal (<25%) staining.

259 Fascin Expression Is Associated with Triple Negative (Basal-Like) Invasive Mammary Carcinoma and an Aggressive Phenotype

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Background: Basal-like mammary carcinoma is an aggressive sub-type identified initially by gene profiling and accounts for 15-30% of all breast cancers. These tumors express basal epithelial markers including CK5, but lack expression of the estrogen receptor (ER), progesterone receptor (PR) and HER2 and are often referred to as triple negative (TN) invasive mammary carcinoma. Fascin is an actin-binding protein, with the ability to facilitate the formation and stabilization of actin protrusions and actin-membrane interactions that mediate cell migration and invasion. Fascin expression has been reported in a number of epithelial malignancies including breast, colon, lung and ovarian carcinoma. In our earlier study we found significant correlation with $\alpha 6\beta 4$ integrin expression in basal-like breast cancer, since $\alpha 6\beta 4$ integrin and fascin share common biological properties, we sought to investigate the expression of fascin in invasive ductal carcinoma with a special focus on basal-like tumors.
Design: We retrieved 99 invasive ductal carcinoma from our surgical pathology files received over a 4-year (1998-2002). All the slides and reports were reviewed to confirm the diagnosis and establish tumor characteristics such as grade, lymph node metastasis, size, ER, PR, and HER-2 status of all tumors. CK5/6 immunostaining was also available on these cases. Fascin immunostaining was performed on formalin fixed paraffin

embedded sections using a mouse monoclonal antibody. Fascin expression was graded as positive or negative and positive expression was correlated with tumor characteristics mentioned above. To compare fascin expression in TN tumors with tumors without a basal-like phenotype we used Chi-Square test for data analysis.
Results: Of the 99 tumors, 25 were TN, and 19 of these were positive for CK5/6 on immunohistochemistry. All of these 25 TN (basal-like) breast cancers were positive of fascin as opposed in the remaining 74 cases that did not show the basal-like phenotype only 27 (36%) were fascin positive ($p<0.0001$). Of the latter 27 cases 11 were CK5/6 positive. In addition fascin positive tumors tended to be associated with larger tumor size, higher grade with evidence of necrosis.
Conclusions: Fascin expression significantly correlates with triple negative (basal-like) invasive mammary carcinoma. Our data also indicates that there are some fascin-positive tumors that are not basal-like therefore it will be informative to assess the prognosis of patients with such tumors as a function of fascin expression.

260 What Is the Relationship between Closely Approximated Low-Grade Ductal and Lobular Lesions in the Breast? A Molecular Study of 10 Cases

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Background: The evolutionary relationship between well-differentiated/low-grade ductal and lobular carcinoma is ambiguous, and is highlighted in cases where ductal and lobular preinvasive and invasive carcinomas coexist in close proximity to one another. Buerger et al (*Mol Pathol* 2000;53:118) hypothesized that such lesions represent the proliferation of a single clone with two different morphological appearances. We tested this hypothesis by analyzing loss of heterozygosity (LOH) in cases with coexistent ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS) and invasive carcinoma.
Design: Ten cases of coexistent low-grade DCIS, classical type LCIS and invasive carcinoma were studied. The invasive component was well-differentiated ductal carcinoma (IDC, 6 cases), moderately-differentiated IDC (3 cases) or lobular carcinoma (ILC, 1 case). Laser capture microdissection was used to isolate DNA from the separate components, which were present either on the same slide or in closely adjacent tissue. LOH analysis was performed separately on each component using 8 commonly deleted markers (6 on chromosome 16q and 1 each on chromosomes 1 and 17). Normal allelotypes were derived from normal lymph node tissue.
Results: In 7 cases, including 5 of 6 lesions containing well-differentiated IDC, the coexistent DCIS and LCIS showed loss of the same allele in at least 1 of the 8 loci interrogated. The IDC component shared loss of the same allele in 4 of these cases, although it usually contained additional LOH patterns not seen in the LCIS or DCIS components. Coexistent DCIS and LCIS often exhibited additional, non-shared LOH as well. The single case of coexisting DCIS, LCIS and ILC showed identical allelotypes in the LCIS and ILC, different from that of the DCIS, implying a collision event involving two separate clones.
Conclusions: Shared LOH patterns suggesting clonality were found between coexistent LCIS and DCIS in 7 of 10 cases, and among LCIS, DCIS and IDC in 4 cases; these results are consistent with the model of Buerger et al. Non-shared LOH among the components indicates that the evolution between LCIS and DCIS may potentially be bi-directional, or that they might be derived from a common precursor but have evolved separately. Since the possibility of LCIS evolving into ductal carcinoma cannot be excluded in this scenario, our results would advocate reporting of localized LCIS at surgical margins in the setting of adjacent low-grade ductal carcinoma.

261 The Significance of Quantity and Histologic Pattern of Atypical Ductal Hyperplasia (ADH) Diagnosed on Core Needle Biopsy of the Breast: Analysis of 118 Cases with Subsequent Excision

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Background: Atypical ductal hyperplasia (ADH) diagnosed on core needle biopsy (CNB) is currently regarded as an indication for surgical excision, as approximately 25% of excisional biopsies will reveal the presence of a more significant lesion (i.e. in situ or invasive carcinoma). We investigated whether histologic features and extent of ADH in CNB specimens are predictive of the presence of in situ or invasive carcinoma on subsequent surgical excision.
Design: One hundred eighteen CNB cases with a diagnosis of ADH and subsequent surgical excision were identified between 2000 and 2007. All cases were reviewed and the diagnoses confirmed. The extent of ADH in CNB was assessed by determining the number of large ducts and/or terminal duct lobular units affected. The extent and histologic subtype of ADH in CNB were correlated with findings on the subsequent surgical excision specimens.
Results: The indication for CNB was calcifications, architectural distortion detected on mammography and palpable mass in 105, 12 and 1 cases, respectively. The median number of cores was 9 (range 2 - 26). Of the 118 cases ADH was restricted to ≤2 foci in 81 (68.6%) and involved more than 2 foci in 37 (31.4%) cases. The predominant histologic subtype was cribriform in 77 (65.3%), micropapillary in 32 (27.1%) and solid in 9 (7.6%) cases, respectively. The corresponding findings at excision were benign lesions without atypia (n=55, 46.6%), focal residual ADH (n=42, 35.6%), atypical lobular hyperplasia (n=2, 1.7%) and ductal carcinoma in situ (DCIS) (n=19, 16.1%). When the number of foci of involvement by ADH on CNB was correlated with the excisional biopsy results, ADH present in more than 2 foci was a strong predictor of a more advanced lesion (DCIS) on excision (13 of 37 vs 6 of 81 cases, p=0.0003, Fisher's exact test). All 6 patients with ADH in ≤2 foci in CNB diagnosed with DCIS on excision had residual microcalcifications after CNB. When histologic subtype of ADH was evaluated, we found that micropapillary subtype significantly predicted the presence of a more significant lesion (p=0.0034, Chi-square test).
Conclusions: Our study suggests that histologic subtype (micropapillary ADH) and the extent of ADH in CNB can be applied to predict the presence of a more significant lesion on subsequent surgical excision. Patients with ADH restricted to ≤2 foci may not need surgical excision, especially if the mammographic abnormality has been completely removed by CNB.

262 Loss of Heterozygosity of PTEN in Pregnancy-Associated Breast Cancer and Its Correlation with BRCA1/2, p53, NM23, Her-2 and Hormonal Status

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Background: Breast cancer is the second most common cancer during pregnancy. Affected patients usually present with large tumor masses, lymph node metastasis and advanced stage. Phosphate and tensin homolog (PTEN) is a tumor suppressor gene associated with aggressive tumor behavior. Studies have suggested that dysregulation of PTEN contributes to disease progression and hormone resistance. We evaluated the presence of LOH in the PTEN gene and its role in tumor progression in cases of breast cancer associated with pregnancy. Correlation with other prognostic factors was done.
Design: Forty-five cases of pregnancy-associated breast cancer were studied. Ninety five percent were infiltrating ductal carcinoma and 4 percent had features of inflammatory carcinoma. DNA obtained from microdissected (FFPE) tissues of 16 cases was used to analyze LOH on different microsatellites for PTEN, BRCA 1/2, TP53 and NM23 genes. LOH results were correlated with other prognostic markers: TP53, ER, PR and HER2, as well as the clinico-pathologic characteristics to further define the effects of the genomic changes on the clinical outcome of patients.
Results: PTEN gene (D10S1173) was informative in 66%cases, and 44% had LOH. All cases showing LOH were ER, HER-2 negative, and 33% were PR negative. The most frequent LOH was in the TP53 region (87%). LOH for BRCA1 (D17S855) was found in 33.3 % of the cases and correlated histologically with a higher nuclear grade and advanced stage. The microsatellite D13S290 was the only BRCA2 marker to show LOH (25%) and no correlation with the clinico-pathologic variables was found. For NM23, 28.5% of the cases showed LOH. Follow-up ranged from 3 months to 13 years. 33% of patients had DOD at 6.5 years, 44% had NED up to 26 years, and 33% were AWD up to 11 years.
Conclusions: Patients with breast cancer during pregnancy are younger, have larger tumors and present with advanced disease. Our findings confirm that dysregulation of the PTEN occurs frequently in their tumors. LOH in the PTEN gene in these patients seems to have an important correlation with poor prognostic markers.

263 RNA Binding Protein IMP3 Is a Novel Biomarker for Basal-Like Invasive Mammary Carcinoma and Is Associated with a More Aggressive Phenotype

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Background: IMP-3 an oncofetal protein is a member of the insulin-like growth factor-II (IGF-II) mRNA-binding protein (IMP) family. Its relevance as a biomarker in lung, pancreatic, renal and cervical carcinoma was recently revealed. However its role in breast carcinogenesis and tumor progression is not yet established. Basal-like mammary carcinoma initially identified by gene profiling accounts for 15-30% of all breast cancers. These tumors express basal epithelial markers including CK5, but lack expression of the estrogen receptor (ER), progesterone receptor (PR) and HER2. They have been found to be associated with a worse overall and disease-free survival. In this retrospective study we examined the IMP3 expression in invasive ductal carcinoma (IDC) of the breast and correlated its expression with established prognostic factors and survival.
Design: The study group comprised of 149 cases of IDC received over a 5-year period between 1997 and 2001. Survival data and clinical stage was available on 132 patients, with a mean follow-up of 58.1 month. Tumor characteristics including size, grade, lympho-vascular invasion, necrosis, lymph node metastasis, ER, PR and HER2 status was obtained from pathology reports. Immunohistochemistry was performed on formalin fixed, paraffin embedded tissue using mouse monoclonal antibody against IMP3 and cytokeratin 5/6 (CK5/6). Statistical analysis was performed using Fishers exact test for correlating IMP3 expression with tumor characteristics and Cox proportional-hazard model was used to perform multivariate analysis for the overall survival.
Results: Of the 149 breast cancer cases IMP3 expression was seen in 55 (37%). 48 of the IMP3+ cases were ER/PR and HER2 negative (triple negative) with basal-like phenotype. Fishers exact test found significant correlation between IMP3 expression and higher tumor grade ($p = 0.001$, necrosis ($p < 0.0001$) and all markers of basal-like breast carcinoma (absence of ER, PR and HER-2 and presence CK5/6 expression) ($p < 0.0001$ for each). Cox multivariate analysis showed a hazard ratio of IMP3 expression at 3.13 ($p=0.05$).
Conclusions: IMP3 is a novel biomarker for triple negative (basal-like) invasive mammary carcinoma and its expression is associated with more aggressive phenotype and decreased overall survival. IMP3 expression may be useful as a prognostic marker and help in the management of breast cancer.

264 Characterization of Estrogen Receptor Positive Breast Cancers in *BRCA1* Mutation Carriers

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Background: Breast cancers in *BRCA1* mutation carriers are usually estrogen receptor (ER) negative (-) and more than 80% have a basal-like molecular phenotype. These tumors are typically poorly differentiated invasive ductal carcinomas with a high mitotic rate and frequently show a prominent lymphoid infiltrate, pushing or circumscribed margins, and geographic necrosis or a central fibrotic focus. However, some women

with *BRCA1* germline mutations develop ER positive (+) cancers; little is known about the characteristics of the ER+ tumors in this group.
Design: We identified 19 ER+ carcinomas among 86 invasive breast cancers that developed in women with *BRCA1* germline mutations (22%). The histologic features of 16 of these tumors with available pathologic material for review were analyzed in detail and compared with those of 18 previously studied ER- cancers that developed among *BRCA1* mutation carriers.
Results: Mean patient age was 48y for ER+ and 43y for ER- cases. The ER+ cancers were histologically diverse. Ten were invasive ductal, 2 invasive with ductal and lobular features, 1 mucinous, and 3 microinvasive in association with DCIS. Two (12.5%) were grade I, 3 (18.7%) grade II and 8 (50%) grade III (in the 3 cases with microinvasion, invasive foci were too small to grade). In contrast, all ER- tumors were grade III infiltrating ductal carcinomas. Of note, among the 8 grade III ER+ cancers, only one showed morphologic features typically associated with grade III ER-, *BRCA1* tumors. Moreover, none of the grade III ER+ tumors had geographic necrosis or a central fibrotic focus, and only one case showed pushing margins and a prominent lymphoid infiltrate (compared with 50%, 44% and 83% of the ER- tumors, respectively). Furthermore, only 55% of the ER+ cancers had a brisk mitotic rate compared to 100% of the ER- tumors.
Conclusions: To our knowledge, this study is the first to document in detail the histologic features of the uncommon ER+ breast cancers occurring in *BRCA1* mutation carriers. Our observations suggest that ER+ breast cancers in *BRCA1* mutation carriers represent a morphologically diverse group, akin to sporadic breast cancers, raising the possibility that at least some ER+ breast cancers that develop in women with germline *BRCA1* mutations may be sporadic rather than *BRCA1*-associated. We are currently analyzing these lesions with a panel of biomarkers to further address this important issue.

265 Impact of Additional Margins on Breast Conserving Surgery in Patients with Ductal Carcinoma In Situ

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Background: Ductal carcinoma in situ (DCIS) is typically treated with breast conserving surgery followed by radiotherapy. There is wide variation in BCS techniques. This study aims to correlate rate of re-excision with BCT with concurrent additional margin excision.
Design: Patients who underwent surgery at NYUMC for DCIS between January 2004 and July 2007 were identified and pathology reports were reviewed.
Results: 405 patients who underwent surgery for DCIS were identified. Table 1 shows clinicopathological characteristics of the patients, surgical technique, DCIS extent, grade, necrosis, the rate of re-excision and residual disease. Of 405 patients, 201 were treated with breast conservative surgery without additional margins (BCS), 151 were treated with BCS with additional margins (BCSAM), and 53 with total mastectomy (TM). Margins were positive for DCIS in 43 (21%) of 201 patients treated with BCS, and in 17 (11%) of 151 patients treated with BCSAM ($P<0.05$); margins were widely negative (> 10 mm) in 36 (18%) of 201 patients with BCS, and in 60 (40%) of 151 patients with BCSAM ($P<0.0001$). Re-excision for close or involved margins was performed in 129 (64%) treated with BCS, and in 61 (40%) treated with BCSAM ($P<0.0001$). Re-excision was performed in 190 of the total 405 patients and a total of 98 patients had residual disease, of which 65 patients were treated with BCS and 33 ($P<0.05$) patients with BCSAM. Presence of DCIS < 1 mm from the margin, was associated with residual disease ($P<0.05$). The rate of re-excision was significantly higher when DCIS was close to 3 or more margins in the original surgery ($P<0.01$). Invasive carcinoma was found at re-excision in 5 patients (4 invasive ductal carcinoma, 1 invasive lobular carcinoma), all of which were previously treated with BCS.

Table 1

		BCS	BCSAM
#		201	151
Age (mean)		58	59
Grade	I	17	10
	II	88	53
	II	96	88
Size (cm)	<1	67	44
	1-2	37	22
	2-5	27	19
	>5	9	1
	N/A	61	65
Margin (mm)	Positive	43	17 *
	<1	73	44
	1-2	23	9
	3-5	20	17
	6-9	6	4
	>=10	36	60 ***
# of margins involved	1-2	92	59
	>=3	73	32
Re-excision		129	61 ***
Residual DCIS at re-excision		65	33 **

*P<0.05; **P<0.01; ***P<0.001

Conclusions: Surgical technique significantly impacts the rate of re-excision in patients with DCIS. BCS with additional margins submitted separately is less likely to lead to a re-excision for residual DCIS.

266 Characterization of the Expression of p-S6rb Protein, a Key Mediator of the mTOR Pathway, in Invasive Carcinomas of the Breast

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Background: The mammalian target of rapamycin (mTOR) pathway is considered a central regulatory pathway involved in cell cycle progression and tumorigenesis. Activation of mTOR leads to phosphorylation of eIF4E-binding protein-1 (4EBP-1) and ribosomal p70S6 kinase. The latter results in phosphorylation of the S6 protein (p-S6rb) in the 40S ribosome subunit initiating protein translation and cell cycle progression. Rapamycin and its analogs inhibit the mTOR pathway and are being tested in clinical trials as novel-targeted anticancer agents. Although it appears that HER2/neu overexpressing tumors may be more sensitive, there are currently no predictive factors that may assist in the selection of which patients may respond to rapamycin or its analogs. The purpose of this study is to characterize the expression of p-S6rb in breast cancer as a marker of mTOR activation and determine whether p-S6rb is associated with HER2/neu overexpression.
Design: P-S6rb expression was determined by immunohistochemistry on 160 largely consecutive invasive carcinomas with long follow-up, using a high-density tissue microarray. Three 0.6 mm tissue cores per cancer were represented per array to account for tumor heterogeneity. Tumors with <20% cells showing strong cytoplasmic p-S6rb staining or tumors showing only weak staining were scored as 1+. Tumors with strong cytoplasmic p-S6rb expression in 21-50% of cells were scored as 2+ and those with >50% cells staining as 3+. HER2/neu status was scored following the DAKO system (0-3+).
Results: Of the 160 invasive carcinomas, 149 had available cores for evaluation. Expression of p-S6rb was present in 90% of the invasive carcinomas (1+ in 37%, 2+ in 37% and 3+ in 16% of cases) indicating activation of mTOR. P-S6rb was present in the cytoplasm of the cancer cells in a diffuse pattern. There was a significant association between strong (2+ and 3+) p-S6rb expression and HER2/neu overexpression (t test $p=0.006$). In preliminary statistical analysis p-S6rb expression was independent of tumor grade and size.
Conclusions: Our study characterizes the expression of p-S6rb in a large cohort of invasive carcinomas of the breast. p-S6rb is a cytoplasmic protein whose activation is associated with HER2/neu overexpression. Our study opens the way to the investigation of a possible mechanistic link between mTOR and HER2/neu pathways, and sets the foundation for the evaluation of p-S6rb as a predictor of rapamicin sensitivity in breast cancer patients.

267 The Effect of Mitosis Marker Phospho-Histone H3 (Ser10) on the Grading of Invasive Ductal Carcinoma

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Background: Mitotic activity is one of three components of the Nottingham grading system for invasive breast cancer. However, accurately counting mitotic figures is challenging because routine methods are time consuming and subject to problems of reproducibility. Phospho-Histone H3 Ser 10 (PHH3) is a recently identified antibody that specifically identifies cells undergoing mitosis. In this study we compare mitotic counts obtained by routine light microscopy with those obtained using PHH3 in a series of invasive breast cancers. We evaluate whether the PHH3 mitotic count results in a shift in Nottingham mitotic score and overall grade. In addition, we assess its impact on prognosis.
Design: 100 consecutive cases of invasive ductal carcinomas in lumpectomy/mastectomy specimens accessioned between 1/1/2000 and 11/8/2000 were analyzed. 18 cases were eliminated due to insufficient tissue. Clinical follow-up was available in 81/82 patients. Using routine methodology, the mitotic count, Nottingham mitotic score, and overall Nottingham grade were determined. Tumor sections were stained for PHH3 (Upstate Cell Signaling Solutions) and PHH3 positive mitotic figures were counted. The new mitotic counts, mitotic score, and overall grade were recorded and compared with those derived by routine methods.
Results: Mitotic counts using PHH3 were significantly higher than those obtained from routine methods (median number 12.5 vs 4.0, $p<0.001$). Implementing the PHH3 stain increased both the mitotic score and overall Nottingham grade (see below).

Routine versus PHH3 Mitotic Score

Score	Routine	PHH3
1	45 (54.9%)	22 (26.8%)
2	16 (19.5%)	12 (14.6%)
3	21 (25.6%)	48 (58.6%)
Total	82	82

$p<0.001$, grade 3 vs grade 1&2, routine vs PHH3, bi-variable GEE logistic regression

Routine versus PHH3 Overall Grade

Grade	Routine	PHH3
1	26 (31.7%)	22 (26.8%)
2	30 (36.6%)	19 (23.2%)
3	26 (31.7%)	41 (50.0%)
Total	82	82

$p<0.001$, grade 3 vs grade 1 & 2, routine vs PHH3, bi-variable GEE logistic regression
Conclusions: The PHH3 immunostain improved our ability to identify mitotic figures in breast cancer. The technique resulted in a significant increase in Nottingham mitotic score and overall grade. Tumors that were raised from a low grade to a high grade with the PHH3 method showed a trend toward a greater rate of recurrence, although this was not statistically significant ($p=0.190$) and may require longer follow-up to assess.

268 Attenuated D2-40 Staining Pattern in Breast Myoepithelial Cells: A Potential Caveat in the Diagnosis of Lymphatic Invasion

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Background: Immunohistochemistry (IHC) for D2-40, which is used to support the diagnosis of lymphatic invasion in breast carcinoma (BC), has also been shown to highlight myoepithelial (ME) cells. Although in most cases such cells can readily be distinguished from lymphatic endothelium (LE), we recently reviewed a case in which

an attenuated D2-40 staining pattern in ME cells led to an incorrect interpretation as lymphatic invasion. This prompted us to further investigate the D2-40 expression patterns in ME cells and LE.
Design: IHC was performed on 42 sequential cases of BC to characterize the pattern and intensity of D2-40 staining in LE and ME cells, including those in non-neoplastic breast tissue and in foci of ductal carcinoma in-situ (DCIS). Cases with presumed lymphatic invasion (tumor cells within a D2-40-positive space) were further characterized based on review of hematoxylin and eosin (H&E) sections and results of additional endothelial (CD31, CD34) and ME (p63, calponin) markers.
Results: Normal lymphatic vessels displayed a consistently strong (3+) linear D2-40 staining pattern, while the ME layer in non-neoplastic breast tissue was characterized by a moderate to strong (2-3+) branching membranous pattern. The ME layer in most foci of DCIS [24 of 26 (92%)] displayed weaker (1-2+) D2-40 staining with significant attenuation of the membranous pattern, including 4 (15%) cases showing a linear pattern similar to LE. Based on D2-40 immunostaining, there were 10 foci of presumed lymphatic invasion. Upon review of the H&E sections and additional IHC, only 6 could be confirmed, while 3 proved to have surrounding ME cells and were thus interpreted as foci of DCIS; one case remained equivocal.
Conclusions: (1) In most cases the pattern of D2-40 staining in LE can be easily distinguished from that in ME cells; (2) however, ME cells in DCIS can occasionally display an intensity/pattern of staining similar to LE. (3) Accordingly, in the evaluation of lymphatic invasion, one should always interpret results of D2-40 staining in the context of the H&E appearances and consider performing additional IHC for endothelial and/or ME markers, especially when the characteristic strong linear pattern of LE staining is not evident.

269 Magnetic Resonance Imaging (MRI) Guided Breast Core Biopsy (CNB): A Pathologic Correlation

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Background: MRI guided core needle biopsy (CNB) is a recently used modality for breast cancer diagnosis. We characterized the pathological features of MRI guided CNB's, and correlated them with patients' clinical histories and MRI findings.
Design: All MRI-guided CNB's were retrieved from our pathology database dated from 1/2005 to 9/2007. The pathological findings were grouped as carcinoma (insitu and invasive), atypical changes (ADH, radial scar, intraductal papilloma) and benign findings (BF). We compared the frequency of CA, atypical changes (AC) and BF in various years, and correlated with the patients' clinical findings.
Results: The median patient age was 56 years (26-84 yr). Of 261 cases, 58 patients had recently diagnosed breast carcinoma (CCa), 59 had remote breast carcinoma (RCa), 13 had a family history of breast carcinoma (FH), and 131 had no available clinical history (NH). Of the 261 cases, 189 (72.4%) were benign (133 fibrocystic changes/apocrine metaplasia, 19 cases were fibroadenoma and 37 others), 24 (9.2%) cases were AC (6 ADH, 18 radial scar/papilloma), and 48 (18.4%) cases were malignant (22 infiltrating and 26 in situ). In 2005, 2006, and 2007, CA were found in 12.0%, 15.3% and 15.7% of biopsies, respectively, whereas AC was found in 4.0%, 8.1% and 14.6% of biopsies, and BF in 84.0%, 76.6%, and 69.7% of the biopsies. Carcinoma was identified in 29% (17/58) of patients with CCa, 20.3% (12/59) of patient with RCa, and 14.5% (19/131) of patients with NH. AC was identified in 11.5% (15/131) of patients with NH, 8.5% (5/59) with RCa, and 6.9% (4/58) with CCa. In 137 enhancing masses, 98 (71.5%) were diagnosed as BF, 12(8.8%) as AC, and 27(19.7%) as CA. In 44 inderterminate masses, 35 (79.5%) were BF, 2 (4.5%) AC, and 7 (9.8%) CA. The diganoses of 12 spiculated masses was 7 (58.3%) BF, and 5 (41.7%) CA. Fifty one cases with suspicious enchancements on MRI correlated with 38 (74.5%) BF, 8 (15.7) AC, and 5 (9.8%) CA. Patients with positive FH and negative mammogram had benign findings on MRI.
Conclusions: A gradual decrease of BF (false positive) and an increase of AC was noted from 2005 to 2007 (84% vs 70%), possibly related to experience and a learning curve. Our data also indicated that 20-29% patients with CCa or RCa and suspicious MRI findings have CA on MRI CNB. FH (in the absence of mammographic/sonographic findings) was not helpful for MRI screening. MRI CNB has a low positive rate, which may be improved by experience, radiologic-pathologic correlation and accumulation of patients with CCa, RCa and other risk factors.

270 Immunohistochemical Evaluation of Multiple Stromal Markers in Fibroadenoma and Phyllodes Tumor

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Background: Both fibroadenoma (FA) and phyllodes tumor (PT) are known to share certain histologic similarities. The stromal cells in both lesions are neoplastic, however they have not been well characterized immunhistochemically with a panel of stromal markers related to fibroblastic, myofibroblastic and myoepithelial differentiation.
Design: Forty-seven cases of mammary fibroepithelial tumors were histologically classified to 23 FA [9 ICFA, 9 pericanalicular FA (PCFA), 2 mixed and 3 juvenile FA (JFA)] and 24 PT (15 benign, 8 borderline and 1 malignant). Immunohistochemical stains for calponin, P63, CD34, CD10, D2-40, F13a, and β-catenin were performed on paraffin sections and immunoreactivity was scored as product of the intensity (1+, 2+, 3+) multiplied by the extent (%) of the positive staining.
Results: Calponin, p63, D2-40 and CD10 highlighted the myoepithelial cells in all cases. The stromal cells in both FA and PT were uniformly positive for calponin and negative for p63. F13a was only positive in rare dentritic cells. D2-40 stained stromal cell poorly in both lesions but revealed diminished or no intratumoral lymphatic vessels (ITLV) in FA and PT. No blood vessel density change was noted on CD34 staining. The findings of CD10, CD34, β-catenin and ITLV are summarized in Table 1.

Table 1	CD10	CD34	Diminished ITLV	β-catenin
Dx	mean score	mean score	% of cases	mean score
PCFA (n9)	19	72	25	32
Mixed FA (n2)	45	85	0	45
ICFA (n9)	97	137	56	37
JFA (n3)	80	21	33	63
Benign PT (n15)	61	69	67	96
Borderline PT (n15)	57	40	87	71
Malignant PT (n1)	90	0	100	90

Conclusions: The stromal cells of both FA and PT express calponin strongly and uniformly; CD10, CD34 and β-catenin variably and p63, D2-40 and F13a poorly. FA tend to have a CD34>CD10 profile except JFA which has CD10>CD34 profile similar to some of the PT. The diminished ITLV is consistent with the orgin of FA and PT from the lymphatic-poor intralobular stroma and might be a sign of stromal overgrowth as it is most frequently seen in borderline/malignant PF followed by benign PT, ICFA and other FA. The level of β-catenin is low in FA, moderate in JFA and high in PT. The stromal cells of both FA and PT share a similar immunophenotype with gradual transition in expression of CD10, CD34 and β-catenin, and the frequency of decreased ITLV between the two. These findings suggest that FA and PT likely represent two ends of the spectrum of a similar fibroepitheilal process.

271 Factors Associated with the Overexpression of the Independent Prognostic Marker eIF4E in Breast Cancer

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Background: Eukaryotic initial factor 4E (eIF4E) is involved in the initial step of protein translation and a number of observation has been made in the role of eIF4E in the progression of cancer. Overexpression of eIF4E has been reported in several human malignancies, including breast, colon, and head and neck. Our group and others have shown that eIF4E was an independent prognostic factor for breast cancer and the overexpression of eIF4E was not related to lymph node status or tumor stages. The aim of this study is to look for the relationship between overexpression of eIF4E and tumor size, tumor grade, tumor type, patient's race/ethnicity, and hormone receptor status.
Design: Consent, clinical data, and tumor specimens were accrued prospectively from 292 patients with stage I-IV breast cancer per institutional reviewed board-approval protocol. eIF4E was quantified for each tumor by western blot analysis and the eIF4E level was divided into three groups: low (<7.5 folds), intermediate (7.5-14 folds), and high (>14 folds). Chi-square and t-test were used for statistical analysis.
Results: Total 292 female patients were included in this study with an age of mean 60 year-old (ranging 29-97). There were 116 white, 175 African-American, and 1 Asian. The tumor types included 252 infiltrating ductal carcinoma, 19 infiltrating lobular carcinoma, 11 medullary carcinoma, 3 mucinous carcinoma, 4 tubular carcinoma, 2 metaplastic carcinoma, and 1 micropapillary carcinoma. The tumor stages were 67 stage I, 142 stage II, 73 stage III, and 8 stage IV, and the tumor grades were 6 grade I, 174 grade II, 110 grade III, and 2 unknown. eIF4E was overexpressed in all breast cancer cases ranging from 2-46 folds comparing with normal breast tissue. The high expression (>14 folds) of eIF4E was significantly associated with tumor size ($p<0.004$) but not related to tumor grade, tumor type, hormone receptor status, or patient's race/ethnicity.
Conclusions: Large tumor size was correlated with high eIF4E overexpression but not tumor grade, tumor type, hormone receptor status, or patient's race/ethnicity. Our previous work has demonstrated that eIF4E was correlated with increased microvessel density. Thus one possible reason for our current finding may be that high eIF4E overexpression is a tumor response to hypoxia.

272 Breast Density, Calcifications, and Breast Cancer Subtypes among Premenopausal Women

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Background: Mammographic breast density is one of the strongest known risk factors for breast cancer among young women. Risk factors for different subtypes of breast cancer may be distinct. The risk of triple negative breast cancer is higher for premenopausal women. We conducted a study with the objectives of evaluating whether mammographic breast density was associated with the risk of different breast cancer subtypes among premenopausal women and whether prevalence of mammographically detected calcifications differed by breast cancer subtypes.
Design: This study benefits from an already existing cohort of women (n=815) diagnosed with their first primary invasive breast cancer. Women were categorized by phenotypic status of ER, PR, and HER2 as: 1) ER+/PR+/HER2-, 2) ER+/PR+/HER2+, 3) ER-/PR-/HER2+, and 4) ER-/PR-/HER2-. Breast density was classified by using of the 4 categories in the [Breast Imaging Reporting and Data Coding System]: 1) almost entirely fat, 2) scattered fibroglandular density, 3) heterogenously dense, and 4) extremely dense. Radiographically detected calcifications were classified as benign, probably benign, suspicious, and malignant.
Results: Among the members of the cohort, 183 were premenopausal. Phenotypic classification yielded a total of 30 (17.1%) women with ER-/PR-/HER2-, 11 (6.3%) with ER-/PR-/HER2+, 90 (51.1%) with ER+/PR+/HER2-, and 46 (25.6%) with ER+/PR+/HER2+. Mammographic density of the majority (n=114, 62.3%) was classified as heterogenously dense. The breast density of 47 (25.7%) and 21(11.5%) was classified as scattered fibroglandular density and extremely dense, respectively. About 50% of women diagnosed with ER-/PR-/HER2+ were mammographically diagnosed as having extremely dense breasts compared with 8.1% of women with ER+/PR+/HER2- and 4.6% with ER+/PR+/HER2+. None of the women diagnosed with ER-/PR-/HER2- had extremely dense breasts ($p=.069$). Calcifications were detected in 74 women. Of these, 18 were benign, 9 probably benign, 28 suspicious, and 19 malignant. The prevalence

of malignant appearing calcifications was the highest among women diagnosed with ER+ /PR+/HER2+ (55%) followed by women diagnosed with ER-/PR-/HER2- (18%) (p=.032).

Conclusions: Among premenopausal women, extremely dense breast may be a risk factor for ER-/PR-/HER2+ subtype but not ER-/PR-/HER2- subtype. The prevalence of "malignant appearing calcifications" was the highest among ER+/PR+/HER2+ subtype followed by ER-/PR-/HER2- subtype.

273 Immunohistochemical Characterization of Hormone Receptor Negative (Triple Negative and HER2+) Breast Carcinomas

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Background: Hormone receptor negative breast cancers are focus of attention due to poor outcome. The histologic features overlap between ER-/PR-/HER2- tumors (triple negative, TNT) and ER-/PR-/HER2+ tumors (HER2+), with poorly differentiated grade as hallmark for both. Majority, but not all, TNT are regarded as basal-like based on HMWCK expression. It is not well known if some ER-/PR- tumors express androgen receptor (AR). We compared the immunohistochemical profile of TNT and HER2+ tumors for basal-like differentiation, AR expression, proliferative activity, and biologic behavior.

Design: 181 ER-/PR- breast cancers from 2001 to 2005 were grouped as TNT and HER2+ based on HER2 status (IHC +/-FISH). Immunostains performed on tissue microarray blocks were: CK5/6, CK8, AR, MIB-1, BCL-2, p53, C-KIT, Cyclin D1, and Vimentin. Cytoplasmic stain in >10% of the tumor cells was scored as + for CK 5/6, Vimentin, CK8, BCL-2, and C-KIT. Nuclear stain in >10% of tumor cells was scored as + for AR, p53, Cyclin D1, and MIB-1. Follow up data was collected for all cases.

Results: Among 181 tumors, 142 were TNT (78.5%, 67 node+, 22 mets, 14 deaths, 2 recurrences); 39 were HER2+ (21.5%, 18 node+, 5 mets, no death). CK 5/6 positivity was more frequent in TNT, 34 (23.9%, 12 node+, 9 mets) vs 1 (2.6%, 1 node+, no met) in HER2+ ($p<0.05$). Vimentin was more frequently positive in TNT, 54 (38.0%) vs 5 (12.8%) in HER2+. AR was rarely positive in either group, 1 (0.7%) and 2 (5.1%) respectively in TNT and HER2+. CK8 positivity was similar between TNT (46, 32.4%) and HER2+ (20, 51.3%). Increased p53 and MIB-1 expression was seen in both groups, 60 (42.2%, 21 node+, 19 mets) and 97 (68.3%, 25 node+, 20 mets) respectively in TNT, 16 (41.0%, 6 node+, 1 met) and 30 (76.9%, 13 node+, 5 mets) respectively in HER2+. BCL-2 positivity was seen in 21 (14.8%) TNT and 1 (2.6%) HER2+ ($p<0.05$). Cyclin D1 was detected in 8 (20.5%) HER2+, higher than those in TNT (6, 4.2%). HER2+ tumors were negative for C-KIT (0%), in contrast to TNT (20, 14.1%) ($p<0.05$).

Conclusions: 1). CK5/6 positive tumors were typically seen in TNT (23.9%) with exception of 1 in HER2+ group and were associated with adverse outcome. 2). AR was rarely positive in ER-/PR- tumors. 3). CK8 was positive in about half of tumors in both groups. 4). Increased proliferative activity (MIB1) and p53 mutations were seen in both groups and were associated with adverse outcome. 5). C-KIT, BCL-2, and cyclin D1 expression differences between two groups were not associated with adverse outcome.

Cardiovascular

274 Migration of Human Umbilical Cord Blood Monomuclear Cells for the Treatment of Acute Myocardial Infarction

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Background: Previous studies indicate that Human umbilical cord blood mononuclear progenitor cells (HUCBC) injected into infarcted myocardium of rats within 2 h or at 24 hours after left anterior descending coronary artery (LAD) occlusion resulted in significantly smaller infarction sizes 1 month later than the control injected saline group of rats. Experiments showed not only limitation to the infarct size at 2 and 12 hour post ligation. Herein we explore the both the localization of stem cells with in the infarcted myocardium as well as their tropism for specific tissues.

Design: Source of HUCBC: Cryopreserved (-196°) mononuclear fractions of HUCBC were given by Saneron CCEL Therapeutics, Inc. The LAD was permanently ligated in 4 rats, with 10 x 6 HUCBC in 0.5 ml of saline directly injected at the edge of the infarction zone at the apex as soon as the infarcted area could be seen. In two of the rats 0.5 ml of saline was injected at the edge of the infarction zone as soon as it was seen after ligation (roughly around 30-45 minutes). All the rats were evaluated for the presence of stem cells in the spleen, thymus and liver in both the control and treated lines along with any histological abnormalities. Stem cells were enumerated at 60x with the average whole number reported from 10 consecutive non-overlapping fields. Human stem cells were enumerated with the aid of the following immunohistochemical antibodies: CD117, CD-34 and HLA-A All slides were evaluated with adequate negative and positive controls by a single board certified pathologist.

Results: HLA-A (+), CD-117(+), CD-34 (+) HUCBC were seen within and adjacent to infarcted myocardium 4/HPF, as well as the spleen 6/HPF, thymus 12/HPF, and liver 2/HPF. The lung, brain, thyroid, pancreas, and soft tissues were essentially devoid of HUCBC. The spleen demonstrated stem cells at the red/white pulp interface, and the thymus showed preferential location at the cortical/medullary interface. Stem cells within the liver were present uniformly within the portal regions.

Conclusions: Stem cell homing to infarcted myocardium was demonstrating confirming previous studies. Significant numbers of stem cells were unexpectantly observed within the reticuloendothelial organs including the thymus, spleen, and liver. Interestingly, the stem cells were seen preferentially within the latter organs at the interfaces between B and T-cell lymphocytes zones. The mechanism(s) that underlie these observations are unknown yet could involve dendritic cell interaction of cytokine/surface receptor homing.

275 Pathologic Features of Hypertrophic Cardiomyopathy in Exertional and Non-Exertional Sudden Deaths

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Background: The pathologic features that characterize hypertrophic cardiomyopathy (HCM) in exertional vs. non-exertional sudden deaths have not been extensively studied.

Design: We prospectively performed gross measurements and histologic analysis on 107 autopsy cases of HCM and correlated them with clinical findings.

Results: There were 107 cases, separated into four groups: exertional sudden death (n=38), non-exertional sudden death (n=36), non-sudden deaths in patients with known HCM (n=14), and incidental HCM in patients dying of non-cardiac cases (n=19). Pathologic features of the 74 sudden deaths were compared between exertional and non-exertional deaths. Age at death was significantly lower in exertional (26.6 ± 13.6 years) vs. non-exertional sudden deaths (42.7 ± 15.0 years, $p<.0001$). There was no significant difference in the incidence of syncope in the exertional sudden deaths (27%) compared to the non-exertional sudden deaths (26%, p=0.8), or in the rate of a prior diagnosis of HCM (13% vs. 17%, respectively). The proportion of women was significantly less in the exertional sudden death group (7.9%) vs. the non-exertional sudden death group (36%, p=.01). The mean heart weight in men was significantly less in the exertional sudden deaths (521 ± 169 g) vs. the non-exertional sudden deaths (698 ± 190 g, $p<.001$). There was no difference in the proportion of hearts with septal: free wall ratios >1.3 (43%) in exertional vs. non-exertional (43%) sudden deaths, in macroscopic septal scarring (15% vs. 15%), or intramural coronary dysplasia (37% vs. 42%). There was a non-significant increase in myocardial bridging >3 mm (21 vs. 13%, p = 0.6) and left ventricular outflow tract plaque (58 vs. 38%, p=.06), respectively. By multivariate analysis, including all categories of HCM, only age (p=.002) and heart weight (p=.02) were significantly associated with exertional sudden death, both in an inverse relationship.

Conclusions: There are no pathologic features which would identify patients with HCM at risk for exertional death. Because young age and relatively low heart weight are strongly associated with exertional death, and because a high proportion of exertional sudden deaths with HCM are not associated with significant asymmetry, cardiologists should be careful in excluding the diagnosis of HCM in athletes with even mild degrees of cardiomegaly, especially young males.

276 Myocarditis in Arrhythmogenic Right Ventricular Cardiomyopathy Due to Desmosomal Gene Mutations: Is There an Infective Etiopathogenesis?

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Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disease characterized by a gradual loss of myocytes and fibro-fatty replacement. Recently, mutations of gene encoding desmosomal proteins have been demonstrated in up to 50% of probands. Inflammatory infiltrates are identified in two-thirds of cases as to support an infective etiopathogenesis. The aim of this study was to assess the presence of viral genomes in the myocardium by molecular pathology investigation on hearts of genotyped ARVC patients.

Design: Ten ARVC hearts (8 male, 2 female, mean age 28 yrs), coming from either sudden death (7) or cardiac transplantation (3) were investigated. Genetic screening identified pathogenetic mutations in plakophilin-2 (5 cases), desmoplakin (3), desmoglein-2 (1) and plakoglobin (1). After gross examination, extensive sampling of both ventricles and septum was performed for histology and immunohistochemistry. Paraffin-embedded or formalin fixed myocardial samples were analysed by polymerase chain reaction for the presence of cardiotropic viruses, including adenovirus, herpes virus, influenza virus A and B, hepatitis C, enterovirus and parvovirus.

Results: At macroscopic examination, there was biventricular involvement in all (predominantly right in 3 and left in 1). At histology, fibro-fatty replacement with inflammatory infiltration were evident in all (100%). The latter was either diffuse (3, 30%) or focal (7, 70%), and mostly consisted of T-lymphocytes in 8 (80%) and was polymorphous in 2 (20%). Clear-cut evidence of myocyte necrosis was present in 3 (33%). Nucleic acids extraction was adequate in 9 (90%). Molecular investigation was negative in all but 1 case in which HCV was identified (10%)

Conclusions: Myocarditis is a usual feature in genotyped ARVC hearts, which are characterized also by biventricular involvement and fibrofatty replacement. On the opposite, viral genome is a exceptionally detected in the myocardium as to question a causative role of viruses and to support the view of myocarditis as a reactive phenomenon accompanying the injury and repair process of ARVC.

277 Morphologic Findings of Coronary Culprit Lesions in Premature Familial Sudden Coronary Death

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Background: The morphologic features of premature familial coronary artery disease are not known. The presence and type of coronary thrombus may have important implications in the genetic basis for familial heart disease.

Design: Autopsies of sudden coronary death (SCD) victims over a 5-year period from a statewide medical examiners office were studied. Coronary arteries were sectioned at 3-5 mm and every segment with narrowing of >50% was submitted for histologic evaluation. Familial disease was defined as sudden death at ≤50 years in women and ≤45 years in men, with premature SCD or acute coronary syndrome in a first-degree relative. Culprit lesion was defined as acute plaque rupture, plaque erosion, and severe narrowing without thrombus (stable plaque).