containing a variety of soft tissue neoplasms. The assay employs probes specific for *MDM2* (12q15, Vysis®) and the centromere of chromosome 12 (*CEP12,* Vysis®). After counting signals from 40 nuclei/case we calculated a *MDM2/CEP12* ratio; a ratio >2.0 was considered amplified, ≤2.0 non-amplified, and ~1 with >2 signals of both probes polysomy for *CEP12*.

Results: 96.4% of WDLS/ALT and DDLS showed amplification of *MDM2* (mean: >17 copies/nucleus; *MDM2/CEP12* ratio: 7.5), with amplification in both the atypical and cytologically unremarkable cells, and the degree of amplification correlating to the degree of cytologic atypia. *CEP12* polysomy was noted in 8/9 (88.9%) SC/PL (*MDM2/CEP12* ratio: 0.97). All angiolipomas and lipomas were non-amplified (*MDM2/CEP12* ratio: 1). 1/1 epithelioid sarcoma, 1/4 malignant peripheral nerve sheath tumors, and 1/5 myxoid liposarcomas showed *MDM2* amplification. All other soft tissue neoplasms were non-amplified.

Conclusions: The *MDM2/CEP12* FISH assay is a sensitive and specific tool (93% and 100%, respectively) for evaluating atypical lipomatous neoplasms. The specificity decreases when evaluating HGS, since *MDM2* amplification was seen in a subset of HGS other than DDLS. In most instances, these other HGS can be distinguished from DDLS by their immunohistochemical profile or characteristic translocation (i.e. t(12;16) involving CHOP in MLS). No benign lipomatous lesions were *MDM2* amplified and the non-atypical cells in WDLS were positive, making the probe a valuable diagnostic tool in the differential diagnosis of WDLS/ALT and DDLS.

79 D2-40 (Podoplanin) Is a Novel Marker for Follicular Dendritic Cell Sarcoma

H Yu, JA Gibson, GS Pinkus, JL Hornick. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Background: Podoplanin is a membrane glycoprotein expressed in a variety of human cell types, including glomerular epithelium, lymphatic endothelium, and mesothelium. Recent studies have demonstrated the utility of podoplanin (recognized by monoclonal antibody D2-40) in the diagnosis of mesothelioma and seminoma. In the course of evaluating clinical cases, we observed that follicular dendritic cells (FDC) within lymphoid follicles are strongly positive for D2-40. However, podoplanin expression in FDC sarcomas has not previously been examined. The purpose of this study was to evaluate the expression and specificity of podoplanin in FDC sarcomas compared to other spindle cell sarcomas.

Design: 80 tumors were studied: 11 FDC sarcomas; 29 gastrointestinal stromal tumors (GIST), including 13 spindle cell, 7 epithelioid, and 9 mixed type; and 10 cases each of malignant peripheral nerve sheath tumor (MPNST), leiomyosarcoma, monophasic synovial sarcoma, and solitary fibrous tumor (SFT). Immunohistochemical studies were performed following epitope retrieval (0.01M citrate buffer, pH 6.0; pressure cooker) using monoclonal antibody D2-40 (Signet Labs) and an EnVision+ peroxidase detection system (Dako). The extent of immunoreactivity was graded according to the percentage of positive tumor cells: 0, <5%; 1+, 5-25%; 2+, 26-50%; 3+, 51-75%; and 4+, 76-100%; and the intensity of staining was graded as weak, moderate, or strong.

Results: All FDC sarcomas (100%) showed moderate or strong immunoreactivity for D2-40 (4+ [5], 3+ [2], 2+ [4]). D2-40 expression was only occasionally observed in the other tumor types examined: 7 (24%) GISTs (4+ [1], 3+ [3], 2+ [1], 1+ [2]), 3 (30%) synovial sarcomas (3+ [1], 2+ [1], 1+ [1]), 1 (10%) MPNST (1+), 1 (10%) leiomyosarcoma (3+), and 0 (0%) SFT. Interestingly, immunoreactivity for D2-40 was more common in spindle cell GISTs (38%) than in epithelioid or mixed-type GISTs (13%). In contrast to FDC sarcomas, the intensity of staining for D2-40 was weak in all but 5 of the other positive tumors.

Conclusions: Podoplanin is a highly sensitive marker for FDC sarcoma and may be useful in a panel for characterization of these unusual tumors. However, a subset of GISTs (in particular, spindle cell type) is also positive for D2-40, and limited podoplanin expression may occasionally be detected in other spindle cell sarcomas.

Breast

80 Morphological and Molecular Evolutionary Pathways of Low Grade Breast Cancers and Their Putative Precursor Lesions

T Abdel-Fatah, DG Powe, Z Hodi, JS Reis-Filho, A Lee, IO Ellis. Nottingham University, Nottingham, United Kingdom; Institute of Cancer Research, London, United Kingdom.

Background: We investigated the morphological and molecular evolutionary pathways involved in the development of low grade breast cancers by studying the frequency of association and immunoprofile of putative precursor lesions including CCLs, UEH, ADH, DCIS, and LN.

Design: The frequency of invasive and pre-invasive lesions was microscopically determined in 200 low grade breast tumours comprising low grade IDC, cribriform, pure and mixed type tubular (TC), tubulolobular (TLC) and ILC classic type. Tissue microarrays containing 1100 of these lesions were immunohistochemically compared for putative tumour suppressor genes (TSGs), cell cycle regulators, proliferation and differentiation markers.

Results: Morphology: > 85% low grade IDC, cribriform, TC and TLC had associated CCLs with the majority showing flat epithelial atypia (FEA). CCL, DCIS and invasive lesions co-localized in >80% patients; LN occured in only 16% cases compared to 91% ILCs. Immunohistochemistry: Epithelial cells in the putative precursor FEA, ADH, LN, DCIS lesions and associated cancers were negative for basal/ myoepithelial markers but positive for CK19/18/8, ER-α, Bcl-2, and Cyclin D-1. Contiguous cells expressing ER-α increased in columnar cell hyperplasia (CCH), rising with atypia. ER-α/ER-βratio and Cyclin D-1 expression increased during carcinogenesis. Bcl-2 was frequent in epithelial cells lining CCLs, ADH, LN and low grade DCIS compared to associated carcinoma. FHIT and ATM expression was reduced in hyperplastic CCLs, ADH/DCIS, LN and associated invasive lesions.

Conclusions: We suggest that CCLs, particularly FEA, are common in low grade breast carcinoma and ILC, representing a family of precursor, *in situ* and invasive neoplastic lesions belonging to the luminal 'A' subclass. Our results suggest that the committed progenitor cells for low grade breast neoplasia are CK19/18/8+, ER-α and Bcl-2+. The balance between ER-α/ER-β expression may be important in driving cyclin D-1 and Bcl-2 expression. Alternatively, 'breast cancer stem/ progenitor cells' regardless of their original phenotype acquire early stochastic genetic/epigenetic hits, leading to activation of the luminal A pathway (ER/cyclin D1 pathways) that determine the phenotype of pre-invasive and invasive lesions. We speculate that once cells commit to this 'molecular pathway', progression to a 'high grade' (basal-like or HER2) phenotype would be unlikely.

81 Intra- and Peritumoral Lymphatic Vessel Density in Breast Cancer: Correlation with Clinicopathologic Features and Prognostic Significance

G Acs, X Xu, T Pasha, PJ Zhang. H. Lee Moffitt Cancer Center, Tampa, FL; University of Pennsylvania Medical Center, Philadelphia, PA.

Background: The earliest feature of disseminated disease in breast cancer is regional lymph node involvement. Despite its major role in tumor dissemination, little is known about the interaction of tumor cells with lympahtic vessels, the role of tumor lymphangiogenesis in the metastatic process and whether lymphatic spread occurs via pre-existing lymphatics or vessels newly formed by lymphangiogenesis. The monoclonal antibody D2-40 detects a fixation-resistant epitope on podoplanin and has been shown to be a selective marker for lymphatic endothelium useful in specifically identifying lymphatic vessels in malignant neoplasms.

Design: We examined the intra- and peritumor lymphatic vessel density (LVD) in a series of 256 pT1 and pT2 invasive breast carcinomas using D2-40 immunohistochemistry on formalin-fixed paraffin-embedded tissue sections. The lymphatic density within the tumors (intratumor LVD, ILVD) and within 2 mm of the edge of tumors (peritumor LVD, PLVD) was determined in 5 high power fields (X200) withy the highest number of D2-40 positive lymphatic vessels. The ILVD and PLVD was correlated with clinicopathologic tumor features, the extent of retraction artifact around tumor cell nests and patient outcome.

Results: Intra- and peritumoral lymphatic vessels were detected in 116 (45%) and 249 (97%) tumors, respectively. Peritumor LVD (5.64 ± 0.28, mean ± SEM) was significantly higher compared to intratumor LVD (0.86 ± 0.11) ($p < 0.0001$). High intra- and peritumor LVD showed significant correlation with tumor grade, tumor size, presence of lymphatic invasion, nodal metastasis, extensive retraction artifact around tumor cell nests and presence of D2-40 immunoreactivity in tumor stroma. High intra- and peritumor LVD was highly significantly associated with poor recurrence-free and overall survival in univariate analysis. In multivariate analysis, high peritumor LVD remained an independent factor predicting poor recurrence-free survival.

Conclusions: High density of both intra- and peritumor lymphatic vessels in breast carcinomas are associated with more advanced, agressive diasease and poor outcome, suggesting that tumor associated lymphangiogenesis may play an important role in the progression of breast cancer. Determination of LVD may serve as a prognostic and/or predictive factor in breast cancers.

82 Effect of Preoperative Chemotherapy (PCT) on Locally Advanced Breast Cancer (LABC) among Mexican Women

I Alvarado-Cabrero, M Patiño, A Hernandez, S Barroso, E Ruiz. Mexican Oncology Hospital, Mexico, DF, Mexico.

Background: Preoperative chemotherapy (PCT) is a therapeutic option for locally advanced breast cancer(LABC). Pathological assessment of response to PCT in each patient could be use as an in vivo method to assess chemotherapy sensitivity. **Goals**: To evaluate the effects of chemotherapy(CT) on breast cancer tissue and to know the factors that can influence in the pathologic response.

Design: Between july 2000 and june 2003, 135 patients presented to the Hospital with LABC. All patients had a biopsy proven diagnosis of breast carcinoma. Chemotherapy consisted of 4-6 cycles of preoperative FEC, followed by mastectomy with axillary lymph node dissection(ALND). A complete pathologic response(PCR) was defined as the absence of any microscopic evidence of tumor in the mastectomy specimen and ALND. Noncomplete pathologic response(NPR) was defined as having invasive carcinoma at excision. Immunohistochemical stainings(estrogen receptor(ER), progesterone receptor(PR) and c-erB-2) were performed on 135 pretherapeutical specimens. Fisher´s exact test was used for statistical analysis.

Results: The patient's ages ranged from 24 to 79years with a mean age of 54 years. Eleven of the 135(8%) patients had a PCR while 3(2%) patients had microscopic residual disease in lymph nodes. Patient age failed to predict the clinical or pathologic response of breast tumors(P: 0.8). The mean tumor size was 6cm; initial tumor size was not a predictor of PCR(P: 0.6). Of the 135 invasive carcinomas, 90(67%) were ductal(IDC), 31(23%) lobular (ILC), 12(9%) pure micropapillary carcinoma (IMPC) and 2(1.4%) mucinous carcinoma. Thirty five(42%) IDC diagnosed on the biopsy turned out to have two or three types of tumor.(eg.tubular)in the mastectomy specimen. Seventy nine of the cases were grade II and 56 grade III. Patients with a PCR had initial tumors that were more likely to be anaplastic(P:0.3). A PCR was predictive of a axillary lymph node response(P: 0.1). IMPC had a high incidence of ALNMetastases (m:11, Lymph nodes). ER, PR, and Her-2/neu expression was not associated with response to PCT.

Conclusions: PCR among Mexican patiens with LABD is low(8%) High incidence of ILC, no cases of local response IMPC is an aggressive tumor resistant to conventional CT.

83 Intraoperative Sentinel Lymph Node Evaluation Leads to Disparity in Treatment of Breast Cancer Patients

AB Ambaye, A Ciampa, S Naud, DL Weaver. UVM, Burlington, VT.

Background: Intraoperative sentinel lymph node (ISLN) evaluation is routinely used to identify patients with positive SLN and these patients are usually treated with immediate completion axillary lymph node dissection (cALND). The false negative rate for ISLN is high leading to two groups of node positive patients: first detected on ISLN versus first detected on permanent sections. In the present study, we evaluate the clinical management and outcome of breast cancer patients undergoing cytologic ISLN evaluation.

Design: A series of SLN biopsies performed on 622 clinically node negative, T1 and small T2 breast cancer patients was examined. 506 (81.4%) patients had ISLN evaluation and 116 patients had no ISLN. 90 SLN positive cases were evaluated (39 ISLN positive and 51 permanent section only positive). The cALND rate was determined for each group. Size and number of SLN metastasis, total number of SLN removed, tumor characteristics (tumor type, differentiation, stage), prognostic factors (estrogen and progesterone receptor, Her2-neu status), and patient age were also evaluated.

Results: Immediate cALND was performed in all patients with positive ISLN (3-ITC, 10-micromet, 26-macromet). In contrast, only 13 of 51 (25.5%) patients with SLN positive on permanent sections only underwent cALND (2-ITC, 5-micromet, 6-macromet) and 38 patients did not undergo cALND (6-ITC, 18-micromet, 14-macromet). Comparative statistical analysis was undertaken between patients with (13) and without (38) cALND to identify possible reasons for the disparity in surgical management. No statistically significant single variable was identified. Although statistically not significant, patients that underwent cALND were more likely to have SLN macrometastasis, fewer SLN removed on initial biopsy, and better differentiated tumors.

Conclusions: Our data suggest that: 1) when a positive SLN is detected on ISLN, cALND is generally performed and is a unifactorial decision; 2) when a positive SLN is first detected on permanent section, cALND is a multifactorial decision and many patients/clinicians omit cALND; 3) patients should be informed that omitting ISLN and delaying until all tumor and SLN characteristics are known on permanent section may spare them cALND; 4) SLN positive well differentiated tumors are less likely to receive chemo/radiation and more likely to have cALND; 5) ISLN should be performed only for high risk patients. This approach would further lower ALND rates, in keeping with the original objective for SLN biopsy.

84 Immunohistochemical Phenotypes of Invasive Breast Carcinoma with Negative Axillary Lymph Nodes

FI Aranda, G Peiró, E Adrover, J Seguí, M Niveiro, M Planelles, C Alenda. Hospital General de Alicante, Alicante, Spain.

Background: Subtypes of invasive breast carcinoma (BC) based on the expression of estrogen and progesterone receptors (ER/PR) and Her2 provide information of prognostic and predictive value. In patients with IBC and negative axillary lymph nodes (LNN), the frequencies of the different phenotypes, their correlation with pathological factors, p53 and bcl-2 expression, as well as the outcome, have not been extensively studied.

Design: A total of 380 cases of LLNBC were retrieved from the Surgical Pathology files, at the General Hospital of Alicante (Spain). Median follow-up was 78.2 months (range 6-239 months). Histologic grading was determined according to the Elston-Ellis modified criteria. Immunohistochemical (IHC) staining was performed for ER, PR, Her2, Ki67, p53, bcl-2 and E-cadherine. Tumors were classified as: (a) luminal A (RE/RP-positive, Her-2-negative, Ki67<20%); (b) Luminal B (RE/RP-positive, Her2-negative, Ki67≥20%); (c) Her2 -positive (3+, 2+ confirmed by FISH); (d) basal-like (RE/RP/Her2-negative) and (e) lobular (E-cadherin negative). Significant associations were identified using Chi-square and Fisher´s exact test. Multivariate analysis was determined by Cox´s proportional hazard model. Survival was calculated by the Kaplan-Meier method (log rank test). The level of significance was set at 0.05.

Results: Based on IHC data, 197 (52%) tumors were luminal A, 39 (20%) luminal B, 52 (14%) Her2, 68 (18%) basal-like and 25 (6.6%) lobular. 23% were grade 1, 37% grade 2 and 40% grade 3. Tumors of basal-like and Her-2 were more frequently of grade 3 (85% and 62%, respectively) compared with luminal A (21%), luminal B (49%) or lobular (9%) (p=0.000), with p53 overexpression (p=0.000), bcl-2 negative (p=0.000) and Ki67 ≥20% (p=0.000). Basal-like and Her2 phenotypes, Ki-67 ≥20% and high histological grade correlated negatively with patient's survival (all p<0.0001). Multivariate analysis revealed that phenotypes (p=0.02) and Ki67 (p=0.04) were independent predictors of survival.

.

85 Expression of Prognostic Markers in Core Needle Biopsy and Surgical Specimens in Patients with Breast Carcinoma in Neoadjuvant Chemotherapy

FI Aranda, G Peiró, E Adrover, M Niveiro, C Alenda, J Seguí, J Sánchez-Payá. Hospital General Iniversitari, Alacant, Spain.

Background: Core needle biopsy (CNB) is being used for the diagnosis of breast carcinoma (BC). Previous studies have shown that neoadjuvant (preoperative) chemotherapy (NACT) may induce changes in neoplastic breast tissue. The objective of this study was to evaluate the changes in the expression of several prognostic markers in CNB and surgically resected specimens in patients with BC who received NACT. The results were compared with those obtained in the group of patients treated by adjuvant (postoperative) chemotherapy (ACT).

Design: We studied two groups of tumors: (a) 101 cases from patients with NACT (Anthracycline +/- Taxanes) and (b) 267 cases from patients treated first with surgery followed by ACT. Immunohistochemistry (IHC) for estrogen (ER) and progesterone receptors (PR), Ki67 and HER2 was performed in CNB and surgical specimens. Tumors were considered ER/PR positive when the staining was present in ≥10% of the neoplastic nuclei; HER2 overexpression if score 2-3+ (HercepTest); and high proliferative activity when Ki67 was present in ≥20% nuclei. Association between parameters was assessed using concordance coefficient kappa and Chi-square test.

Results: The sensitivity and specificity in CNB using surgical resection as the gold standard are shown in [Table 1] and [2]. In cases with ACT, a good level of concordance was observed for ER, PR and HER2 (kappa=0.65). In contrast, Ki67 showed a moderate level of concordance (kappa=0.45). Regarding cases with NACT, similar levels of concordance were observed for ER, PR and HER2, but not for Ki67 (56.7% in CNB versus 25.6% in the excision; kappa=0.17; p=0.05). The comparison of sensitivity and specificity in cases with and without NACT showed differences in specificity for PR (p=0.025) and in sensitivity for Ki67 (p<0.000), but not for ER or HER2.

Conclusions: In our series of breast carcinoma, the results support that NACT modifies significantly the status of PR and Ki67 between CNB and the corresponding surgical specimen. *Supported by grant FIS 03/1411.*

1. Results for patients with ACT

	Sensitivity	Specificity	kappa
ER	97.4	72.6	0.75
PR	88.7	79.8	0.65
Ki67 (20%)	83.1	61.1	0.45
HER2 (2-3+)	95.6	69.0	0.69

2. Results for patients with NACT

	Sensitivity	Specificity	kappa
ER	92.6	72.7	0.68
PR	86.7	57.1	0.42
Ki67	49.3	33.3	0.17
HER2 (2-3+)	90.0	86.3	0.67

86 Can High Molecular Weight Cytokeratin, CK-903, Differentiate Benign Proliferative Breast Lesions from Atypical Ductal Hyperplasia and Ductal Carcinoma In Situ?

M Aziz, S Troob, S Simpson, C Mills, S Bernik. St. Vincent's Comprehensive Cancer Center, New York, NY.

Background: This study reports the utility of monoclonal antibody CK-903, specific for High Molecular Weight Cytokeratins 1, 5, 10, 14, in the diagnosis of intraductal breast pathology where morphological criteria failed to clearly define the lesion. Reports of the immunoprofile of IDH, AIDH, and DCIS from previous studies served as the standard of comparison for the staining in this study.

Design: Between October 1, 2005 and July 4, 2006 98 cases of intraductal breast proliferations were reviewed at our institution whose diagnoses were indeterminate based on the morphology. These cases were divided into three groups based on a preliminary morphologic diagnosis: favor benign, favor atypical, or favor DCIS. Each case was then stained with CK-903 and the staining was reported as negative, negative with focally positive, weakly positive, positive, and strongly positive. When staining supported the preliminary diagnosis, the final diagnosis remained the same. The morphology was reevaluated in cases where the staining was discordant with the preliminary diagnosis.

Results: Ninty eight cases with indeterminate morphology were identified. The preliminary diagnosis of these cases based solely on morphology included 46 favor benign, 29 favor atypical, and 23 cases of favor DCIS. Of the 46 favor benign, 44 (96%) stained positive or strongly positive, supporting a benign diagnosis. Two (4%) stained negative or negative with focal positivity, supporting the presence of atypia. Review of these 2 cases revealed morphologic features supporting the diagnosis of atypia. Of the 29 favor atypical, 8 (28%) stained positive or strongly positive, supporting a benign ductal or lobular proliferative diagnosis. Upon review of the morphology of these 8 cases, 7 proved to be benign ductal lesions and 1 LCIS. Of the 23 cases of DCIS, 22 (96%) stained negative or negative with focal positivity, supporting a malignant diagnosis. One (4%) stained with mixed reactivity. Review of the morphology revealed a carcinoma in situ with mixed ductal and lobular features.

Conclusions: Staining with CK-903 confirmed the preliminary diagnosis in 87 (89%) cases. In this series, discordant staining results revealed definitive morphologic features that led to a change in the diagnosis of 11 (11%) cases. CK-903 proved a useful clinical tool for evaluating difficult cases of intraductal proliferation. The stain should not however replace morphology as the primary means of evaluating such lesions.

87 Significance of Atypical Columnar Cell Change in Core Needle Biopsies of Breast

S Bandyopadhyay, M Chivukula, DJ Dabbs. Magee Womens Hospital/UPMC, Pittsburgh, PA.

Background: Atypical ductal hyperplasia (ADH) includes a spectrum of changes which fulfill some but not all the criteria for ductal carcinoma in situ (DCIS). ADH is associated with an increased risk for the subsequent development of carcinoma. The incidence of ADH in core needle biopsies (CNB) has been reported to be less than 10%. Columnar cell lesions (CCL) of the breast comprise a morphological spectrum ranging from columnar cell change to columnar cell change with atypia. Recent studies have shown that CCL with atypia (ACCC) are more frequently associated with genetic imbalances and are considered to be precursors to DCIS and invasive carcinoma. The aim of this study is to evaluate the differences in follow up between cases of ADH and ACCC, diagnosed on CNB.

Design: We reviewed the archives of the Pathology Department of Magee Womens Hospital using the LISS system from January 2001 to July, 2006 and extracted the following categories of cases: 1) Total number of breast core biopsies where ADH was the most significant pathology; 2) Total number core biopsies where ACCC was the most significant pathology, 3) Follow up pathology in resection specimens in both categories 1 and 2. The slides from the above cases were reviewed.

Results: 252 cases had ADH and/or ACCC as the most significant pathology, diagnosed during this period. 118 (47%) of these cases were ACCC and 134(53%) were ADH. In the ACCC category, follow up (resection specimens) was available in 85 cases. It was as follows: Benign in 21 (24.7%) cases, ADH in 41 (48.2%), LCIS in 4 cases(4.7%), DCIS in 12 (14.1%) and Invasive carcinoma (IC) in 7 (8.2%). In the ADH category, follow up (resection specimens) was available in 63 cases. It was as follows: Benign in 28 (44.4%) cases, ADH in 27 (42.8%) cases, LCIS in 3 cases(4.8%), DCIS in 3 (4.8%) and Invasive carcinoma (IC) in 2 (3.2%).

FOLLOW UP IN CASES OF ACCC AND ADH

	BENIGN (%)	ADH (%)	DCIS (%)	LCIS (%)	IC (%)
ACCC (F/U=85)	21/85 (24.7)	41/85 (48.2)	12/85 (14.2)	4/85 (4.7)	7/85 (8.2)
ADH (F/U=63)	21/63 (44.4)	27/63 (42.8)	3/63 (4.8)	3/63 (4.8)	2/63 (3.2)

Table 1

Conclusions: In our study, the presence of ACCC appears to confer a higher risk of for the subsequent development of carcinoma (in situ or invasive), compared to ADH. The presence of ACCC, in CNB, should suggest a more active surveillance of these patients.

88 *BRCA2* Deficiency and *EMSY* Amplification; Possible Mutually Exclusive Genetic Events in the Development of Breast Cancer

AL Bane, N Weerasooriya, IL Andrulis, FP O'Malley. Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.

Background: *BRCA1* and *BRCA2* are two highly penetrant breast cancer susceptibility genes implicated in 30-50% of familial breast cancers. Both genes are considered classic tumor suppressors with inactivation or loss of both alleles required for tumorigenesis. In addition, approximately 10% of sporadic breast cancers have lost functional *BRCA1* through a combination of LOH and/or epigenetic silencing. More recently a potential mechanism for *BRCA2* inactivation in sporadic breast and ovarian cancers has been described; *EMSY* a gene located on 11q13.5, interacts with exon 3 of BRCA2 and has been shown *in vitro* to transcriptionally silence it. This implies that *EMSY* amplification is functionally equivalent to a *BRCA2* null phenotype. EMSY amplification has been shown to occur in 9-13% of sporadic breast cancers; however the presence or absence of *EMSY* amplification has not been studied in *BRCA2*-associated cancers. The objective of our study was to determine the frequency of *EMSY* amplification in *BRCA2*-associated and control tumors.

Design: TMAs were constructed using 64 *BRCA2*-associated tumors and 186 age and ethnically matched control tumors accrued through the Ontario Familial Breast Cancer Registry. TMA sections were analyzed for *EMSY* gene status using FISH. TMA sections were additionally analysed for the expression of hormone receptors, HER2/neu protein, basal and luminal cytokeratins.

Results: None (0%) of *BRCA2*-associated tumors were found to have EMSY amplification as compared to 11 (9%) of the control tumors, (p=0.03). Those tumors with EMSY amplification when compared with non-amplified tumors showed no statistically significant differences with regards to tumor grade, hormonal status or expression of HER2/neu, basal or luminal cytokeratins.

Conclusions: We have shown that *BRCA2*-associated tumors do not exhibit *EMSY* amplification, whereas this gene is amplified in approximately 9% of sporadic breast tumors. Those tumors with *EMSY* amplification do not have a distinguishing phenotype as determined by immunohistochemical profiling. These results suggest that BRCA2 deficiency and *EMSY* amplification may be mutually exclusive genetic events on the road to tumorigenesis.

89 Breast Conservative Treatment for Ductal Carcinoma in Situ (DCIS): Correlation of Recurrences with Pathologic Parameters and University of Southern California/Van Nuys Prognostic Index (USC/VNPI)

M Bansal, V Narra, D Maldonado, A Kapke, U Raju. Henry Ford Hospital, Detroit, MI.

Background: Risk factors reliably predicting recurrence of breast DCIS are yet to be identified. Original VNPI is one of the models to streamline DCIS treatment that includes tumour size, grade, and margin distance with a combined score of 3-9. A recent modification is USC/VNPI with age as 4th parameter (scores 1,2,3 as >60yr, 40-60yr,<40yr respectively).

Design: From 1986-2005, 754 patients were treated for DCIS of whom 276 had mastectomy. Clinical and pathological parameters of 432 patients treated with lumpectomy-observation (LO) or lumpectomy-radiation (LRT) with a follow-up of 0.5 to 20.5 years (median 6.18) were analyzed. According to USC/VNPI scores patients were categorized as Group 1: scores 4,5,6; Group 2: scores 7,8,9; and Group 3: scores 10 and 11(no patient with score of 12). 1, 5, and 10-year recurrence-free survival (RFS) rates were estimated using the Kaplan-Meier method (95% confidence interval) for 1) the three VNPI groups, and 2) the two treatment groups. The log-rank test was used to compare the RFS curves.

Results: There were 56 (13%) patients who had a recurrence; 26 were invasive (5.9%) and 30 were DCIS (6.8%). Patients with tumors 16-40mm and >40mm were 2.1 times (P=0.04) and 4.5 times (P=0.03) more likely to have a recurrence compared to tumors <15mm. Although there was no statistically significant association between recurrence rate (RR) and other risk factors (age, margin and tumor grade), the RR was twice as high (20%) if margin was <1mm opposed to a >1mm margin (10%). All 3 recurrences in patients <40yr were invasive (3/15; P=0.53). High-grade tumors also had high rate of recurrence. RFS curves were significantly different across USC/VNPI groups (P=0.03). The 5-year recurrence-free probability was lowest in Group 3 patients (89%, vs. 96% in Group 1 and 99.5% in Group 2). 10-year probability was lowest among Group 1 patients (71%, vs. 90% in Group 2 and 78.5% in Group 3). 5 and 10-year RFS estimates were higher for patients receiving LRT in all USC-VNPI groups. One young patient with extensive DCIS had invasive chest wall recurrence following mastectomy (0.3%).

Conclusions: 1. Patients with USC/VNPI group 3 exhibit extremely high local recurrence rates regardless of irradiation and should be considered for mastectomy. 2. All the three groups have better recurrence free survival following radiotherapy.3. Among the USC/VNPI parameters, tumor size was the most important and statistically significant in this series.

90 Does Size Matter? Comparison Study of the Tumor Size in Breast Cancer in Lumpectomy Specimens

B Behjatnia, J Sim, LW Bassett, N Moatamed, SK Apple. University of California in Los Angeles, Los Angeles, CA.

Background: MRI is being used with increasing frequency for measurement of breast lesions. Size of lesion is essential in staging cancer to determine type and extent of patient management. Size can be overestimated or underestimated changing subsequent course of action. The purpose of this study was to assess accuracy in estimating tumor size by MRI and gross using microscopy as gold standard. Change in T stage of cancer due to such differences was also analyzed.

Design: A retrospective study was done on 33 female patients, ages 30 to 75 years, who underwent MRI of breasts with subsequent lumpectomy from 2002 to 2006 for invasive breast cancer. Size of lesion(s) on MRI and gross were compared with histological size. Percentage of cases where MRI and gross had overestimated or underestimated size of cancer were calculated. Based on maximum invasive tumor dimension from MRI and microscopy, changes in T-stage were determined.

Results: Thirty-five cases were found among 33 patients. Twenty-five (71%) had invasive ductal (IDC) and 10 (29%) had invasive lobular carcinoma (ILC). Tumor size by MRI matched exact histological size in 3%, underestimated 34%, and overestimated 63% of cases. By MRI, T stage was altered 23% of the time, 14% into higher and 6% into lower stage. Tumor size by gross matched exact histological size in 23%, underestimated 63%, and overestimated 14% of cases. By gross, T stage was altered 54% of the time, 43% into higher and 9% into lower stage.

Conclusions: MRI and gross pathology have significant limitations in predicting tumor size compared with microscopy in breast cancer. T-stage is altered markedly on gross compared to MRI. Both are better in predicting tumor size in IDC than ILC. Overall, 23% of MRI and 54% of gross had false predictive values in assessing T-stage. Differences in T-stage may lead to significant differences in management of patients in lymph node negative breast cancers.

Percentages of IDC and ILC overestimated, underestimated, or showing the same size by MRI and gross pathology and the corresponding changes on T-stage of the tumor

MRI		IDC	ILC	T-stage	IDC	ILC
	underestimated	28%	50%	changed	16%	40%
	overestimated	68%	50%	into higher	8%	30%
	same size	4%	0%	into lower	8%	10%
Gross	underestimated	56%	80%	changed	52%	50%
	overestimated	20%	0%	into higher	40%	50%
	same size	24%	20%	into lower	12%	0%

91 Interleukin-8 Expression Does Not Correlate with Estrogen Receptor Status in Human Breast Cancer

M Bendre, R Hunter, D Gaddy, LJ Suva. UT Houston, Houston, TX; University of Arkansas for Medical Sciences, Little Rock, AR.

Background: In recent years significant effort has focused on dissecting the role and prognostic value of tumor derived cytokines in breast cancer. Interleukin-8 (IL-8), a member of the alpha chemokine superfamily has been implicated in tumor progression. IL-8 has been demonstrated to enhance tumor cell proliferation, motility, invasion and angiogenesis, which correlate with metastatic potential. We and others have demonstrated that human breast cancer cell lines with high metastatic potential have increased IL-8 expression compared with cell lines with little or no metastatic potential. *In vitro* studies using human breast cancer cell lines have shown that IL-8 expression is negatively linked to estrogen receptor (ER) status. These studies implicated increased IL-8 expression in ER- breast cancer cell lines with the increased invasive potential of breast cancer cells. In light of these studies, we examined IL-8 expression in breast tumors in relation to ER status.

Design: Tumor tissue samples from 22 ER+ and 15 ER- invasive ductal carcinomas were selected for this study. These tumors were immunostained for IL-8 expression with appropriate positive and negative controls. The intensity of staining for IL-8 was graded on a scale of 0 to 3+ with 0 representing no detectable staining and 3+ representing the strongest staining.

Results:

ER Status	Total cases	IL-8 expression		
		3+	2+	1+
ER+	22	13(59.1%)	8(36.4%)	1(4.5%)
ER-	15	7(46.7%)	7(46.7%)	1(6.6%)

100% of cases examined stained positive for IL-8 expression. No significant difference was observed in the intensity of IL-8 staining between ER+ and ER- cases ($p<0.05$).

Conclusions: These data demonstrate that invasive ductal carcinomas express IL-8 and its level of expression does not appear to correlate with ER status. These observations are contrary to previously published *in vitro* observations which have shown IL-8 expression to be correlated with ER status. Our study highlights the importance of differences between primary tumors and tumor-derived cell lines. The tumor environment *in vivo* differs from that *in vitro,* as these cells are under completely different selection pressures. These data demonstrate that IL-8 expression in primary breast cancer is independent of ER status. In light of our previous observations highlighting the important role of IL-8 in breast cancer progression further investigation is warranted to determine if IL-8 is an independent prognostic indicator in breast cancer.

92 E-Cadherin and P120 Catenin Expression in Basal-Like Invasive Breast Carcinoma

R Bhargava, M Chivukula, GJ Carter, DJ Dabbs. Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: The E-cadherin (E-CAD) complex is composed of the transmembrane E-CAD protein and alpha, beta and gamma p120 catenins (CTN) which anchor the E-CAD protein to the cytoplasmic actin filaments. In addition to the loss of immunodetectable E-CAD in lobular neoplasia, a most characteristic abnormality in lobular neoplasia is the diffuse localization of p120 CTN throughout the cytoplasm, which yields a diffuse cytoplasmic p120 CTN immunostaining pattern. In contrast, ductal neoplasia retains the dominant membrane immunostaining pattern of p120 CTN, reflecting the normal construction of the e-cadherin complex. Basal-like breast carcinoma is a recently recognized subtype in which E-CAD and p120 CTN has not been studied.

Design: Seventeen basal-like breast carcinomas, which have been previously characterized (ER, PR and HER2 negative and positive for basal-like cytokeratins) at our institution were studied for E-CAD and p120 expression by immunohistochemistry (IHC). All cases have shown characteristic morphologic features (high grade, geograpghic necrosis, host lymphocytic response and good circumscription). A tissue microarray with 3-fold redundancy was constructed from these 17 cases. A single 4 microns section was subjected to IHC staining for E-CAD and p120. Based on intensity and percentage of tumor cell staining, the IHC stains were scored from 0 to 4+ for each antibody. In general, a ductal carcinoma shows membranous 3+ or 4+ staining with both E-CAD and p120. A lobular carcinoma usually shows lack of E-CAD and 3-4+ cytoplasmic p120 staining. Therefore reduced expression for E-CAD and p120 was considered when IHC score was 2+ or less.

Results: Of the 17 basal-like carcinomas, 12 (70.6%) showed reduced E-CAD expression. In contrast, only 5 of 17 (29.4%) cases showed reduced p120 expression.

E-CAD versus p120 Expression in Basal-like Carcinoma

	p120 0	p120 1+	p120 2+	p120 3+	p120 4+	Total
E-CAD 0	0	0	0	0	0	0
E-CAD 1+	0	0	2	2	0	4
E-CAD 2+	0	0	3	5	0	8
E-CAD 3+	0	0	0	2	3	5
E-CAD 4+	0	0	0	0	0	0
Total	0	0	5	9	3	17

Conclusions: Most basal-like carcinomas have reduced E-CAD expression; however normal p120 catenin membranous expression is retained, and therefore the E-CAD-catenin complex is preserved. Basal-like carcinomas could be considered a subtype of ductal carcinoma. Reduced E-CAD expression may be one of the reasons for aggressive behavior of basal-like carcinomas.

93 Megakaryocytes in Sentinel Lymph Node May Detract from Identification of Metastatic Breast Cancer or May Be Misdiagnosed as Metastatic Carcinoma

S Bhusnurmath, L Balazs, E Pritchard, N Zafar. University of Tennessee at Memphis, Memphis, TN.

Background: Sentinel lymph node (SLN) from the axilla is frequently submitted for intraoperative consultation to identify metastatic breast carcinoma. Positive SLN results in removal of axillary lymph nodes. Patients of biopsy-proven breast carcinoma often undergo neoadjuvant chemotherapy to improve treatment outcome. The impact of such therapy on nodal microenvironment is not well documented and carries a potential pitfall for surgical pathologists, as highlighted by these cases.

Design: We report three patients (age range: 28-44 years) with biopsy-proven infiltrating mammary ductal carcinoma, status-post neoadjuvant chemotherapy with cyclophosphamide, adriamycin and docetaxel for 4-6 months. The patients underwent lumpectomy (left-sided in two and right-sided in one) with submission of sentinel lymph node/s from ipsilateral axilla for intraoperative consultation. These patients had no hematological abnormality at surgery except mild anemia.

Results: At intraoperative consultation, megakaryocytes were present in the nodal sinuses, with multilobulated hyperchromatic nuclei and abundant eosinophilic cytoplasm. Micrometastasis was present in the first patient and was nearly missed, the second had more easily identifiable metastasis and in the third, tumor cells were identified by immunocytochemistry for cytokeratin. Megakaryocytes were immunoreactive for CD 61 and von Willebrand factor antigen but not cytokeratin.

Conclusions: Possible sources of false-positive interpretation of SLN include misinterpretation of nevus cell aggregates, cytokeratin-positive fibroblastic reticulum cells, plasma cells and multinucleated macrophages. Isolated case reports of megakaryocytes in SLN in patients with breast carcinoma exist (1), but not in the setting of neo-adjuvant chemotherapy. Megakryocytes normally reside in bone marrow, but their presence elsewhere typically denotes extramedullary hematopoiesis. When present in lymph nodes, they average 20-25 um in diameter and are distributed as single cells in nodal sinuses. They may be misdiagnosed as tumor cells in lymph nodes (2), and even if appropriately identified, may induce a false negative diagnosis by distracting the pathologist from identifying coexistent metastatic tumor cells in the intraoperative setting, particularly when the metastatic tumor cells are scarce. 1. Hoda et al. Archiv Pathol Lab Medicine 2002; 126: 618-620, 2. Weeks et al. Human Pathol 1990; 21:1239-1244.

94 Lobular Carcinoma In Situ of the Breast and Breast Imaging; a 10-Year Institutional Review

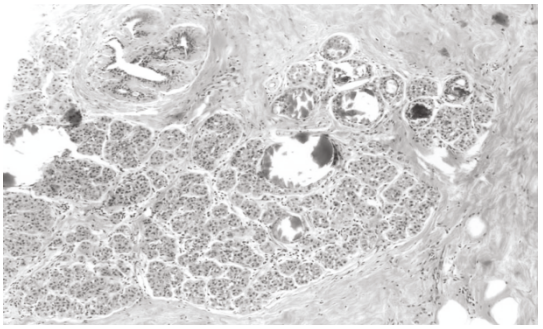
SK Bihlmeyer, DL Weaver. University of Vermont, Burlington, VT.

Background: Lobular Carcinoma in Situ (LCIS) is generally regarded as an incidental microscopic finding, as there are no consistent clinical or radiological findings. The pleomorphic variant of LCIS (large cells with abundant cytoplasm and large eccentric nuclei) has been associated with microcalcifications. Masses and microcalcifications are the primary target of breast biopsies. On occasion, we had noted that LCIS was the most aggressive histologic finding in biopsies performed for mammographic calcifications.

Design: We retrospectively identified all of the cases over a 10-year period (1994-2004) in a large academic hospital in Vermont, where LCIS was the only form of carcinoma identified. A slide review was then performed to qualify the specimens in which the LCIS was identified. The microscopic target of the biopsy specimen was evaluated. If calcifications were present, the extent and type of calcification was evaluated to determine whether the LCIS was associated with the biopsy target.

Results: 51 cases were identified in 47 patients with a median age of 56 years (range, 38-83). The main indications for biopsy were calcifications (n = 27) and mass or lesion (n = 14), or other (n=10). Stereotactic, excisional, and wire-localization biopsies composed the majority of the specimens (76%). In 46 of the 51 cases (90.2%), the LCIS appeared to be a truly incidental finding. Most frequently, the target of the biopsy was calcifications and these calcifications were microscopically identified in benign areas (commonly identified in microcystic alteration of lobules) with adjacent incidental LCIS. Infrequently, in 5 of 51 cases (9.8%), similar calcifications were identified in both LCIS and benign elements and the LCIS appeared to represent the target of the biopsy, suggesting that LCIS instigated or enhanced the formation of microcalcifications.

Conclusions: Our findings support the hypothesis that LCIS has no consistent radiologic marker. LCIS is an incidental finding not associated with or only incidentally associated with microcalcifications. In approximately 10% of cases of pure LCIS, a radiographic correlate (microcalcifications) is identified. No specific histopathologic features were identified in this subset of LCIS.



95 Analysis of Discordance in HER-2 Over-Expressed (3+)/FISH Negative Breast Carcinomas

KJ Bloom. CLARiENT Inc, Aliso Viejo, CA.

Background: Assessment of HER-2 status by IHC or FISH is acceptable for selecting patients for trastuzumab therapy. While there is an assumption that IHC stained slides assessed as 3+ will show gene amplification, re-testing of 3+ tumors as part of clinical trials has shown discrepancy rates as high as 35%. Little data is available on the cause of such discrepancies.

Design: One hundred consecutive breast carcinomas in which a HER-2 immunostained slide was assessed as 3+ and was subsequently assessed by FISH were included in the study. All slides were stained at CLARiENT using the HercepTest kit (Dako) and all submitted blocks were stated to be fixed in 10% neutral buffered formalin. Analysis was performed utilizing image analysis software, (ACIS V2.4, CLARiENT). No reason was given as to why FISH testing was subsequently ordered. FISH testing was performed using the PathVysion kit (Abbott) according to the manufacturer's guidelines. Images were obtained as part of the original signout process and these were reviewed for each tumor.

Results: Fourteen cases (14%) were identified as discrepant, IHC 3+ and a FISH HER-2: CEP-17 ratio less than 2.2. Two cases included specific mention in the IHC report that strong 2-3+ expression was noted in the benign elements and recommended HER-2 assessment by FISH. Four tumors revealed conclusive 3+ expression of HER-2 but no amplication of the HER-2 gene. One of the tumors was stated to have aneusomy with a HER-2:CEP-17 ratio of 1.7 but a mean HER-2 count of 6.3. Re-analysis of the four tumors revealed amplification in three of the four samples including the one previously thought to show aneusomy. One sample contained both in-situ and invasive tumor. In reviewing the images, the in-situ component was clearly 3+ but the invasive component was 0-1+. The FISH was appropriately interpretted as unamplified. Seven tumors did not reveal 3+ expression when the images were reviewed. All showed expression levels ranging from 1-2+ but contained areas of artifactual 3+ expression including foci of retraction, crush and tissue folds.

Conclusions: While 14% of tumors appeared discrepant, only 1 case was truely an IHC 3+/FISH discrepancy. The vast majority of IHC 3+/FISH discrepancies were the result of inappropriate field selection by the pathologist. Retrospective FISH testing to resolve IHC 3+/FISH discrepancies frequently revealed that amplified tumor cells were over-looked on the initial FISH assessment. Pathologists should use increased caution when selecting IHC fields and should re-assess the FISH slide on all discrepant cases.

96 The "MINO" Mouse Model of DCIS Reveals a Genetically Stable Precancer Stem Cell with a Programmed Malignant Potential

AD Borowsky, P Damonte, JP Gregg, QA Chen, LJT Young, JG Hodgson. UC Davis, Davis, CA; UC Davis, Sacramento, CA; UCSF, San Francisco, CA.

Background: The MINO mouse consists of six lines with distinct morphologic phenotypes and behavior, each meeting experimentally defined criteria for "pre-cancer". Specifically, these serially transplanted outgrowths derived from the Tg(MMTV-PyVmT) mouse grow orthotopically in cleared mammary fat pads and consistently progress to an invasive phenotype capble of ectopic growth. Transition to carcinoma has a consistent latency for each line, ranging from 11wks (+/-1.2) to 22 wks (+/- 1.7). Three of the liines also show pulmonary metastatic potential.

Design: MINO tissues were harvested for DNA extraction and cell dissociations. Comparative genomic hybridizaion was performed by BAC array hybridization, and oligonucleotide array hybridization. Cell dissociation, culture conditions and flow cytometry separations have been optimized. Normal mouse mammary tissues, and tissues from invasive carcinomas were harvested for parallel analysis.
Results: (1) Comparative genomic hybridization shows that the pre-cancer and invasive tumors are genetically stable with low level changes including whole chormosome gains in some lines. Other lines show no genomic abnormalities (euploidy). No changes are associated with progression, though occasional spontaneous focal amplificaitons and deletions are detectable. (2) Dissociation of the precancer lesion cells and three dimensional "spheroid" culture of single cells reveals a bipotential for myoepithelial and luminal differentiation and the formation of unique 3D "MINO-spheres" with intermediate features between nomral and carcinoma cells performed in parallel. (3) Flow cytometric sorting for CD49f+/CD24hi/CD29+/CD45-/CD31-/Ter119-/ single viable cells shows enrichment of the cells capable of forming these MINOspheres. (4) Transplantation of a single MINOsphere recapitulates the outgrowth of the precancer morphology and progression to carcinoma.
Conclusions: These data establish a pre-cancer "stem" cell capable of self renwal and multilineage differentiation as the origin of invasive cancer. In the context of this model, these cells have programmed potential for latency and metastasis that does not appear to require sequential genetic "hits" for transformation.

97 Metaplastic Tumor of the Breast with Pure Sarcomatous Phenotype Associated with Ductal Carcinoma In Situ: Analysis and Characterization of Eight Cases

FI Boulos, JR Stumph, NM Granja, ME Sanders, JF Simpson, DL Page. Vanderbilt University Medical Center, Nashville, TN.
Background: The breast is frequently the site of tumors of ambiguous histopathogenesis, often termed metaplastic to reflect their overlapping epithelial and stromal phenotypic characteristics. These tumors are notoriously variable ranging from low-grade spindled epithelial to high-grade sarcomatous lesions with no evidence of epithelial derivation, and spanning cases where histopathogenetic categories and biologic behaviors cannot be assigned with certainty. We identify a subset of these diagnostically challenging tumors sharing common histologic features of potential clinical significance.
Design: From the files of our Breast Consultation Service, 86 tumors with both a sarcomatous component and an in-situ epithelial component were examined, and 8 with similar histopathologic and immunophenotypic features were described.
Results: Patient ages ranged from 37 to 82 (mean 58). Lesions ranged in size from 0.6 cm to 2.0 cm. All were largely well-circumscribed (one arising in papilloma) with a limited (except for one case) intraductal carcinoma component present in all cases, and located at the periphery of the lesion. The majority of the lesions were of low-cellularity with tumors cells present singly rather than in clusters or sheets. Only 2 showed foci of intermediate cellularity. In all cases the sarcomatous cells showed significant pleomorphism with atypical mitotic figures. Also, 4 cases showed metaplastic bone/osteoid, two of which also with fibrocartilage. Benign multinucleated giant cells were present in 4 cases. AE1/AE3, CK 903, orthokeratin, low molecular weight keratin, vimentin, smooth muscle actin and p63 were available in 6 of 8 cases. The sarcomatous component was uniformly positive for vimentin and negative for keratins. p63 and SMA were variable. We have no knowledge of unfavorable outcomes in any of the patients.
Conclusions: Although these lesions have a sarcomatous phenotype and lack evidence of epithelial derivation, their close proximity to DCIS suggests a metaplastic origin. Hence a designation of metaplastic tumor with pure sarcomatous phenotype and associated ductal carcinoma in-situ is suggested. Also, despite the pleomorphic nuclei and atypical mitoses, the small size, circumscription, singly distributed tumor cells within a fibrous matrix that is often associated with metaplastic bone/fibrocartilage, suggest that these metaplastic lesions should be assigned to a low to intermediate grade rather a high grade category.

98 Cyclin D1 Overexpression with CCND1 Amplification Is Correlated with Loss of BTG2 in Estrogen-Receptor Positive Human Breast Cancer

EF Brachtel, H Kawakubo, JB Kish, G Yeo, S Maheswaran. Massachusetts General Hospital, Harvard Medical School, Boston, MA.
Background: BTG2 (B-Cell Translocation Gene) codes for an anti-proliferative protein expressed in normal breast epithelium and lost in 46% of breast carcinomas. Cyclin D1 (CycD1) is a cell cycle regulator with an important role in breast carcinogenesis and possibly linked to resistance to anti-estrogenic therapy. As recently reported, loss of BTG2 expression was significantly associated with overexpression of CycD1 in estrogen-receptor positive (ER+) breast carcinomas. In this study, we evaluated whether CycD1 overexpression in BTG2 negative breast cancer may be associated with CCND1 amplification. We assessed protein expression by immunohistochemistry for BTG2 and CycD1, and amplification of the CCND1 gene by fluorescence in-situ hybridization (FISH).
Design: 34 cases of ER+ invasive breast carcinomas were selected from the files of the Pathology Department. Formalin-fixed, paraffin-embedded sections containing tumor and adjacent uninvolved glands were stained with BTG2 and CycD1 monoclonal antibodies using standard immunoperoxidase techniques with antigen retrieval. Nuclear staining was evaluated according to the Allred scoring system. On cases with CycD1 overexpression, CCND1 FISH was performed with the Vysis LSI cyclin D1 (11q13)/ CEP11 dual color probe following the manufacturer´s instructions. Signals in 60 nuclei were counted and amplification defined as 11q13:CEP11 ratio of > 2.
Results: All cases (n=34) showed CycD1 overexpression, 71% (n=24) showed down-regulation of BTG2. 18% (n=6) showed amplification of CCND1. Among these samples, 83% (n=5) of tumors with CCND1 amplification demonstrated down-regulation of BTG2. Thus a higher percentage of breast cancers with CCND1 amplification and concurrent CycD1 protein overexpression (83%) demonstrated loss of BTG2 compared with those in which BTG2 expression was retained (17%).
Conclusions: CycD1 protein overexpression was associated with CCND1 gene amplification in 18% of ER+ breast carcinomas; this rate is in keeping with previous reports. Within this group of patients, 83% demonstrated CCND1 amplification and CycD1 protein overexpression with associated loss of BTG2, whereas in 17% with CCND1 amplification and CycD1 protein overexpression demonstrated no loss of BTG2 in the tumor. These data suggest that both gene amplification and epigenetic mechanisms may cause CycD1 overexpression in breast carcinomas.

99 Patterns of Nipple Involvement by Breast Carcinoma in Mastectomies

EF Brachtel, JE Rusby, JS Michaelson, BL Smith, FC Koerner. Massachusetts General Hospital, Harvard Medical School, Boston, MA.
Background: Breast conservation is the optimal standard of surgical care for many patients with breast carcinoma. When mastectomy is necessary, the options now include nipple-sparing mastectomy. This study evaluated data on the patterns of occult nipple involvement in a large, prospectively sampled cohort of patients. The value of the retroareolar margin as an indicator of involvement was assessed since this may provide a basis for the decision to proceed with an oncologically safe nipple-sparing mastectomy.
Design: 178 consecutive mastectomy specimens from female patients without grossly recognized nipple involvement (135 therapeutic, 43 prophylactic) were evaluated. Nipple and retroareolar tissue was excised, formalin-fixed and paraffin-embedded. Coronal, hematoxylin-eosin stained sections through the entire nipple were reviewed. Extent of nipple involvement by invasive ductal or lobular carcinoma (IDC or ILC), lymphovascular invasion (LVI), ductal carcinoma in-situ (DCIS) and lobular neoplasia (LN) were assessed, as well as the level at which the nipple was involved (within the papilla, at the level of the skin or in the margin). The section deep to the skin was considered the potential retroareolar en-face resection margin for a nipple-sparing mastectomy.
Results: Nipple involvement by IDC, ILC, LVI or DCIS was noted in 28/135 (21%) of therapeutic and in none of the prophylactic specimens. 20/84 (24%) patients with IDC showed nipple involvement, 4 by IDC, 16 by DCIS, and 4 by LVI (3 nipples had more than 1 type of pathology). 2/21 (10%) patients with ILC had direct involvement of the nipple. LN was present in 9 nipples (5 %), in both therapeutic (n=7) and prophylactic specimens (n=2). In the therapeutic mastectomies with DCIS, nipple involvement was significantly associated with high histologic grade ($P=0.043$ by Chi^2). Nipple involvement by IDC, ILC or DCIS could be predicted by the retroareolar margin with a sensitivity of 0.67 and a negative predictive value of 0.93.
Conclusions: Overall, 74% of nipples from therapeutic mastectomies showed no pathologic abnormality. The most frequent form of involvement was with grade 3 DCIS. Few invasive carcinomas involved the nipple. None of the prophylactic mastectomies showed nipple involvement by IDC, ILC or DCIS but a few cases showed incidental LN. A retroareolar en-face margin may be used to test for occult involvement in patients undergoing nipple-sparing mastectomy.

100 Clinical, Morphologic and Immunophenotypic Characteristics of Primary Breast Carcinomas (BC) with Brain Metastases (BM)

E Brogi, MP Murray, S Patil, M Akram, T Nehhozina, L Norton, A Seidman, LK Tan. MSKCC, NYC, NY.
Background: BM of BC are notable for symptom severity, limited treatment options and generally poor prognosis. Study of the clinical-pathological features of BC with BM could identify features useful for disease prognostication and treatment.
Design: We identified 71 women with BM of BC, for which BC slides were available for review, including 4 lymph node metastases. Clinical information was abstracted from the medical records. We noted BC morphology, tumor type, tubule formation, nuclear grade (NG), mitoses/10 hpf (MIT), presence of a central acellular zone, dense lymphocytic infiltrate, DCIS, necrosis and calcifications. Reactivity for ER, Her-2, androgen receptor, EGFR, vimentin, CK5/6, CK8/18, calponin, p63 and E-cadherin (E-cad) was assayed in a tissue microarray with 32 BC cases. Data analysis used Wilcoxon Mann-Whitney test for continuous variables and Fisher's Exact test for categorical variables.
Results: Patients median age at BC diagnosis was 46 y (range 31-76), with 19 (27%) patients 40 y or younger. BM occurred at median age of 51 y (range 32-82), after median interval of 3 y (range 1 m-21 y). All BC were invasive, with median size of 2.3 cm (range 0.3-11). Ten BC (14%) had intermediate NG, 61 (86%) high NG; 4 (6%) BC had moderate tubule formation, and 67 (94%) had minimal to none. Median MIT were 14 (range 0-148). BC morphology was basaloid in 20 (28%) cases, squamoid in 13 (16%), micropapillary in 4 (6%), papillary in 2 (3%) and focally mucinous in 1 (1.5%); 1 apocrine and 1 E-cad(-) pleomorphic lobular carcinoma were identified; 2 BC were s/p chemotherapy; the rest were E-cad(+) ductal BC with no special features. There were 13 (41%) ER(-)Her2(+) BC; 11 (34%) ER(-)Her2(-); 4 (13%) ER(+)Her2(-) and 3 (9%) ER(+)Her2(+); ER could not be assessed in one Her2(+) BC. MIT were higher in ER(-) over ER(+) BC (18 vs 10; p=0.05) and in Her2(-) over Her2(+) BC (24 vs 9; p=0.02). Six BC were EGFR(+) (19%). No other parameter tested reached statistical significance, but evaluation of more cases is pending.
Conclusions: This study is the first report on the morphologic characteristics of BC with BM. Our results showed that these carcinomas tend to have basaloid or squamoid morphology, and are frequently ER(-), with nearly equal distribution of Her2(+) and Her2(-) cases. These findings could potentially aid in identifying BC that are more likely to develop BM, and possibly lead to targeted treatment planning.

101 The Superficial Margin in Skin-Sparing Mastectomies for Breast Carcinoma: Factors Predicting Involvement and Efficacy of Additional Margin Sampling

D Cao, TN Tsangaris, P Argani. The Johns Hopkins Hospital, Baltimore, MD.

Background: Skin-sparing mastectomy (SSM) with immediate reconstruction is increasingly performed for breast carcinoma (BC) because of improved cosmesis. However, one concern is that not all of the BC is removed at the superficial margin (SM) of the SSM, where benign breast tissue (BBT) intermingles with the dermis of the skin flaps, potentially promoting local recurrences.

Design: We analyzed 171 SSMs (37 for ductal carcinoma in situ [DCIS] and 134 for invasive carcinoma (IC) +/-DCIS) which had separate, oriented, additional superficial margin (ASM) specimens taken at the time of surgery. ASMs were considered the final, true margins. SSMs were evaluated for their volume, skin area, skin/SSM surface area ratio, BC size, BC stage, IC Elston grade, DCIS nuclear grade, vascular invasion (VI), presence of multiple foci of DCIS away from the IC, the number of foci of IC, Paget's disease, hormone receptor status (ER, PR), Her-2/neu status, deep margin (DM) status (positive or negative), SM status (distance of BC from the inked SM, and extent of involvement (EI) [aggregate length of BC within 1mm of the inked SM]). ASMs were evaluated for BC (if any), BC distance to their inked surface, and presence of BBT. A margin was considered positive if BC was at or within 1mm of the inked surface.

Results: Sixty-five of 171 SSMs (38%) had a positive SM; of these, 13 of 65 (20%) had residual BC in ASMs. In contrast, only 1 of 106 SSMs (1%) with a negative SM had residual BC in its ASM ($p < 0.05$). 89 of 171 (52%) ASMs contained BBT. ASM sampling rendered the final true margin negative in 58 (89%) of 65 SSMs with a positive SM. Factors predictive of a positive SM included a smaller SSM volume, a smaller skin area, a lower skin /SSM surface area ratio, presence of multiple foci of DCIS away from the IC, presence of VI, a higher number of quadrants involved by BC, and a positive DM. In SSMs with a positive SM, the presence of multiple foci of DCIS away from the IC in the SSM was the only factor predictive of residual BC in ASMs; neither EI by BC of the SM nor whether the BC was at or within 1mm of the SM were predictive.

Conclusions: SMs in SSMs are often positive. Approximately half of ASMs contain BBT, likely reflecting the difficulty in completely removing breast tissue near the skin flaps in SSMs. ASM sampling effectively decreases positive margins in SSMs, which should diminish the chances of local recurrence.

102 The Incidence of Concurrent Lobular Neoplasia and Columnar Alterations in Breast Tissues

AM Carley, M Chivukula, GJ Carter, R Karabakhtsian, DJ Dabbs. Magee Womens Hospital of UPMC, Pittsburgh, PA.

Background: Columnar cell alterations (CCA) encompass a spectrum of histologic patterns including benign columnar cell change (CCC), columnar cell hyperplasia (CCH) and hyperplasia with atypia. These entities have a molecular kinship with low grade carcinomas [Mod Pathol 2006, 19(3):344]. Lobular neoplasia (LN) is considered to be a risk factor for developing carcinoma, and LN may also behave as a low grade premalignant process. While LN and some CCA may share genetic abnormalities at 17p, there is no appreciable literature that addresses the simultaneous occurrence of these two lesions in either breast core biopsies or breast resection specimens. The objective of this study is to ascertain whether there is a significant association of CCA and LN in breast tissues of different origins.

Design: The archives of the Dept of Pathology, Magee Womens Hospital of UPMC were retrospectively searched from 1998-2006. The goal was to determine the incidence and association, if any, between CCA and LN. Three types of breast tissue cases were examined: 1) LN diagnosed on core needle biopsy (CNB) targeted for suspicious microcalcifications only (BIRADS 4); 2) A survey of 2516 consecutive breast core biopsies over a one year period; (3) 400 consecutive breast carcinoma resection specimens that were analyzed for LN within the vicinity of the carcinoma. LN includes the diagnoses of atypical lobular hyperplasia and/or lobular carcinoma-in-situ. Glass slides were reviewed for the presence of CCA in association with LN in all three groups.

Results: In group 1, 68 consecutive CNB had LN, and these were found to be associated with CCA in 54.4% (37/68) of cases. The CCA were without atypia. In group 2, LN in association with CCA comprised 1.3% (32/2516) of cases of total CNB. In group 3, 13.0% (52/400) cases of CCA were associated with LN.

Conclusions: LN and CCA occur together significantly more frequently in breast lesions that are classified as suspicious calcifications (BIRADS 4) (group 1). While CCA are relatively common, and LN is uncommon, the significant association of the two in tissue targeted for calcifications suggests the possibility that the two may have a common progenitor with different pathways of neoplastic development.

103 Mammography Misses Lobular Carcinomas with Homogeneous Tumor Cell Distribution: A Target for New Screening Techniques

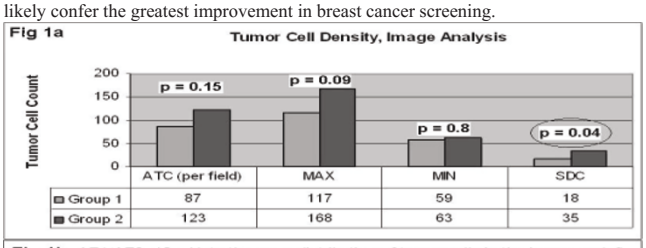
F Chaves, B Burke, A de las Morenas. Boston Univ Med Ctr, Boston, MA.

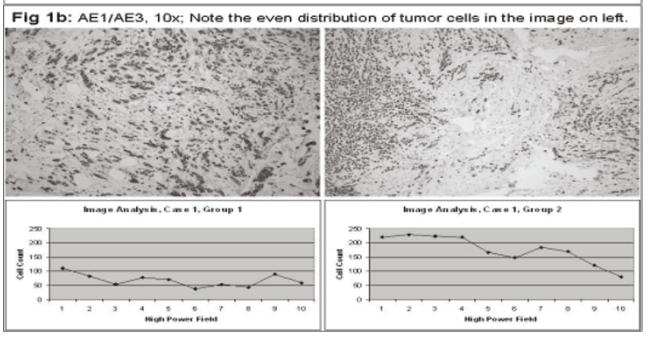
Background: Although mammography is the cornerstone of breast cancer screening, up to 20% of cases have false negative results. Improvements in diagnosis are currently being sought with digital mammograms and breast MRI technologies. Identification of morphologic features that render a tumor undetectable by conventional mammography is crucial, since these may be readily recognized using new and more powerful technologies. Lobular carcinomas (LC) are notorious for their subtle radiologic changes. They also show a wide variation in cellular density, both among tumors and within the same lesion. Therefore, we wanted to evaluate whether the cellular density of LCs was associated with mammographically negative lesions.

Design: Fourteen randomly selected cases of invasive LC were stained for broad spectrum cytokeratin (AE1:AE3). We used Image Analysis (IA) on 10 consecutive high power fields (40x), starting at the most cellular area of the tumor. For each case, we evaluated the average tumor cellularity (ATC), field of maximal cellularity (MAX), field of minimal cellularity (MIN), and Standard Deviation of Cellularity (SDC). The IA operator was blinded to mammogram results. Cases were then separated as follows: Group 1 (n=3, negative mammograms: BI-RADS 1 and 2), and Group 2 (n=11, positive mammograms: BI-RADS 4 and 5). Using the Student t-test, we compared the means obtained in both groups.

Results: Group 1 showed a statistically significant decrease in SDC (Fig 1a), indicating a more homogeneous distribution of tumor cells. There was a marked decrease in ATC and MAX for Group 1, but p values were higher than 0.05, most likely due to the small sample size. The MIN values were similar in both groups, indicating that even Group 2 tumors had areas of sparse cellularity. Examples of both groups are shown in Fig 1b.

Conclusions: A homogeneous tumor cell distribution is the main feature that renders LCs undetectable by mammography. These tumors tend to have a lower overall cellularity and do not show foci of extremely high cellular density. Therefore, new imaging technologies with optimal performance in evaluating smooth hypodense lesions will likely confer the greatest improvement in breast cancer screening.





104 Clinical and Pathologic Features of Ductal Carcinoma In Situ (DCIS) Associated with the Presence of Flat Epithelial Atypia: An Analysis of 441 Cases

L Collins, S Schnitt, N Achacoso, L Nekhlyudov, S Fletcher, R Haque, L Habel. Beth Israel Deaconess Med. Ctr. and Harvard Medical School, Boston, MA; Northern California Kaiser Permanente, CA; Harvard Pilgrim Health Center, Boston, MA; Southern California Kaiser Permanente, CA.

Background: Flat epithelial atypia (FEA) is an alteration of mammary terminal duct lobular units that is considered to be a precursor to or early stage in the development of some forms of DCIS. However, no prior study has systematically evaluated the relationships between clinico-pathologic features of the DCIS and the presence of co-existent FEA. A better understanding of such relationships could provide further insights into the connection between FEA and DCIS.

Design: We performed a detailed slide review in 441 DCIS patients as part of a larger case-control study assessing epidemiologic and pathologic risk factors for local recurrence in women with DCIS treated with breast-conserving therapy. We examined the association between the presence of FEA in these specimens and various clinical factors (age at diagnosis, presentation, family history), pathologic features of DCIS (nuclear grade, architectural pattern, comedo necrosis, cancerization of lobules, stromal desmoplasia, stromal inflammation) and the presence of atypical ductal hyperplasia (ADH), lobular neoplasia (LN), and non-atypical columnar cell lesions (CCL).

Results: FEA was present in 71 (16%) of the 441 specimens with DCIS. The presence of FEA was not significantly associated with age at diagnosis, presentation, or family history of breast cancer. In univariate analysis, pathologic features of DCIS significantly associated with the presence of FEA were low nuclear grade ($p<0.001$), micropapillary pattern ($p<0.001$), absence of comedo necrosis ($p<0.001$), absence of stromal desmoplasia ($p=0.01$) and absence of stromal inflammation ($p=0.01$). In multivariable analysis, the features of DCIS independently associated with FEA were micropapillary pattern ($p=0.02$) and absence of comedo necrosis ($p<0.01$). In addition, FEA was significantly associated with the presence of ADH, LN, and CCL in these specimens in both univariate and multivariable analyses.

Conclusions: FEA is more often seen in association with DCIS lesions with particular pathologic features, such as low nuclear grade, absence of comedo necrosis and micropapillary pattern. These observations provide support for a precursor-product relationship between FEA and DCIS lesions that exhibit such features.

105 Topoisomerase II-Alpha Gene Copy Number Alterations in *ERBB2*-Positive Primary Breast Carcinomas: A Fluorescence *In Situ* Hybridization Study

A Colomer, N Erill, M Górriz, M Verdú, R Roman, R Ibáñez, C Cordon-Cardo, X Puig. Grup Assistència, Barcelona, Spain; Barcelona, Spain.

Background: Topoisomerase II-alpha gene (*TOP2A*) aberrations have been recently pointed as predictive markers of anthracycline-based adjuvant chemotherapy in breast cancer. While coamplification of *ERBB2* and *TOP2A* seems to define a subgroup of high-risk patients likely to benefit from anthracycline treatment, the significance of a

patient having *TOP2A* deletions in the presence of *ERBB2* amplification is still unclear. On the other hand, patients with normal *TOP2A* copy number have been suggested not to take advantage of treatment. In this retrospective study, we investigated the incidence of *TOP2A* genomic alterations in a cohort of primary breast invasive carcinomas previously characterized for *ERBB2* genomic status by fluorescence *in situ* hybridization (FISH).

Design: Our series included 32 formalin-fixed, paraffin-embedded tumors obtained from an equal number of women surgically treated for primary breast cancer in Barcelona, Catalonia. Concerning histopathology, lesions were classified as 29 ductal carcinomas, 2 lobular carcinomas and 1 medullary carcinoma. Assessment of *ERBB2* and *TOP2A* copy number was independently done by dual-color FISH (Vysis) using a common centromeric probe (CEP17) and loci specific probes (HER2 LSI or TOP2A LSI, respectively). Relative copy numbers were calculated as ratios between mean number of LSI signals and mean number of CEP17 signals.

Results: *TOP2A* genomic aberrations were identified in 25 out of 32 *ERBB2*-positive tumors (78%). *TOP2A* was coamplified with *ERBB2* (TOP2A LSI/CEP17 ≥ 2.0) in 15 cases (47%), deleted (TOP2A LSI/CEP17 ≤ 0.7) in 10 cases (31%), while no alterations (0.8 ≤ TOP2A LSI/CEP17 ≤1.9) were found in 7 cases (22%). The mean ERBB2 LSI/CEP17 ratio was 4.6 (range 2.0-10), which was higher than the mean TOP2A LSI/CEP17 ratio of 2.8 (range 2.0-5.4). Seven out of 15 *ERBB2* and *TOP2A*-coamplified tumors exhibited monosomy, whereas 6 were polysomic, and 2 were eusomic. No tumors exhibited monosomy amongst those with *TOP2A* deletions, 6 out of 10 were polysomic, and the 4 left were eusomic.

Conclusions: These results are in agreement with those previously published reporting high incidences of *TOP2A* amplification and deletion within the group of *ERBB2*-amplified breast invasive carcinomas. In our series, FISH allowed identification of 78% *ERBB2*-positive patients likely to benefit from tailored and dose-escalated adjuvant anthracycline-based regimens.

106 Evidence of Evolution and Intrinsic Molecular Relationship in a Spectrum of Morphological Variants of Invasive Lobular Carcinoma with Variable E-Cadherin Status

L Da Silva, S Parry, P Keith, SR Lakhani, P Simpson. University of Queensland, Queensland Institute of Medical Research, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia.

Background: The molecular pathology of classical invasive lobular carcinoma (ILC) and its variants is poorly characterized. E-cadherin expression is lost or reduced in the majority but not in all of ILC. However, there has been an increasing use of E-cadherin immunohistochemistry in clinical practice with some using positive staining to exclude a diagnosis of ILC in favour of invasive ductal carcinoma (IDC). Herein, we have used a combined morphological and molecular analysis to investigate a morphological spectrum of ILC showing mixed E-cadherin immunoreactivity.

Design: Sections were stained with haematoxylin and eosin and subjected to immunohistochemistry for E-cadherin, hormonal receptors (ER, PgR), p53, Ki-67, cyclinD1, HER2. Different morphological components were microdissected and subjected to detailed molecular analysis, including E-cadherin (CDH1) gene sequencing, loss of heterozygosity at 16q22.1 and comparative genomic hybridization (CGH).

Results: Three morphological patterns of ILC were identified; classic, alveolar and solid variants. Small solid clusters of ILC expressed E-cadherin and showed weak positivity for ER, PgR and P53 with low Ki-67 index. In contrast, the classical and alveolar components were E-cadherin and PgR negative with a higher proportion of ER and P53 positive cells and high Ki-67 index. HER2 and cyclinD1 were negative. Despite the variable morphology and E-cadherin status, all three subtypes harboured both loss of 16q and a somatic, inframe deletion of the remaining CDH1 allele (exon 7, 867del24bp). Each also had gain of 1q, amplification of 11p14-11q13 and loss of 11q14-tel. The classic and alveolar areas also harboured 17p-/ 17q+. Further changes (2p-, 6+ and 8+) were present in the alveolar component only, suggesting a progression from solid → classic → alveolar subtypes.

Conclusions: We demonstrated molecular evidence for the clonal evolution of ILC through different morphologies. This progression coincides with loss of E-cadherin expression, altered hormonal status, increasing proliferative index and accumulating genetic changes highlighting the heterogenous nature of ILC. E-cadherin positivity in the solid ILC coincides with gene mutation and loss of the second allele, highlighting the fact that E-cadherin can be expressed despite these mutations. E-cadherin immunohistochemical positivity should not be used to make a diagnosis of IDC.

107 Neuregulin (NRG) Expression Modulates Clinical Response to Herceptin in Patients with Metastatic Breast Cancer (MBC)

E de Alava, M Abad, A Ocaña, CA Rodríguez, JC Montero, JJ Cruz, A Pandiella. Centro de Investigación del Cáncer/Hospital Universitario de Salamanca, Salamanca, Spain.

Background: HER2 is a transmembrane tyrosine kinase whose overexpression has been associated to the genesis/progression of a subset of breast cancers. The function of HER2 may be up-regulated by overexpression, or by the availability of Neuregulins (NRGs), a group of transmembrane polypeptide growth factors. Former in vitro reports have indicated that transmembrane NRGs strongly activate HER2 and cell proliferation in breast cancer cells that do not overexpress HER2. In this cellular model, treatment with Herceptin prevented the proliferative action of transmembrane NRG. This raised the important clinical question of whether patients considered as HER2 negative, but expressing transmembrane NRG, may benefit from treatment with Herceptin.

Design: A retrospective study of 124 patients with early stage or metastatic breast cancer was conducted. Expression of transmembrane NRG was evaluated in this group by immunohistochemistry, and in several patients by Western blot for p-HER2 and NRGs. A subgroup of 32 patients with metastatic breast carcinoma diagnosed and treated with chemotherapy plus Herceptin before 2001 was selected. Statistics were performed to analyze possible correlations between NRG expression and response to Herceptin-based therapies, event-free and overall survival in this subset of patients.

Results: Transmembrane NRG was frequently expressed in breast cancer patients. After evaluating the HER2 status of all patients using FISH and immunohistochemistry (Dako HercepTest), we found that 12 patients were FISH negative and HercepTest negative. Among them, those with high levels of NRG expression had a significantly higher objective response rate to Herceptin-based therapy (partial and complete response). In the group of patients with HER2 amplification high levels of NRG were not associated with a better response. Expression of transmembrane NRG in patients without HER2 amplification correlated a longer event free and overall survival.

Conclusions: We suggest that the spectrum of patients that may benefit from Herceptin-based therapies may be widened to include patients with metastatic breast cancer without HER2 amplification, but expressing transmembrane NRGs. This work is supported by a grant from the Ministry of Education and Science of Spain-FEDER (BMC2003-01192), and funds from the regional Government of Castilla y Leon for the Regional Tumor Bank Network.

108 Decreased Estrogen Receptor and Progesterone Receptor Expression Associated with Neoadjuvant Therapy of Exemestane in Combination with Celecoxib in Postmenopausal Women with Breast Cancer

JR deHart, RE Jimenez, CL Shapiro, SP Povoski. The Arthur G. James Cancer Hospital and Richard J. Solove Research Institute and Comprehensive Cancer Center of the Ohio State University, Columbus, OH.

Background: Exemestane (EXM) is an irreversible steroidal aromatase inhibitor, which is used in the treatment of estrogen receptor (ER) positive breast cancers (BC) in postmenopausal women. However, the long-term effects of EXM on tumor cell expression of hormone receptors is largely unknown.

Design: Samples from postmenopausal women with ER positive BC (stage II, III or IV) who were enrolled in an ongoing phase II study assessing neoadjuvant therapy with EXM and celecoxib prior to definitive surgery treatment were evaluated. All patients underwent a pretreatment (pre-tx) core biopsy prior to receiving 16 weeks of neoadjuvant therapy. A posttreatment (post-tx) specimen was collected at the time of their definitive BC surgery. Sections from both the pre-tx biopsy and the post-tx specimen were evaluated by immunohistochemistry with anti-ER and anti-progesterone receptor (PR). The percent of tumor cells with nuclear staining was recorded for each specimen, as well as the proportion of positive cells combined with the intensity of staining, as per the Allred scoring system. Statistical analysis with ANOVA was performed to compare mean values of the pre-tx and post-tx specimens. A P-value of ≤0.05 was considered statistically significant.

Results: Fourteen patients had pre-tx and post-tx specimens available for analysis. The mean positive cells for ER was 85% (range 50-100%) and the mean Allred score (AS) was 7.6 (range 7-8) for the pre-tx biopsy specimens, as compared to 63% (range 5-95%) and 6.4 (range 4-8) for the post-tx specimens (P=0.032 and 0.006, respectively). The mean positive cells for PR was 52% (range 1-100%) and the mean AS was 6.5 (range 4-8) for the pre-tx biopsy specimens, as compared to 15% (range 0-75%) and 2.7 (range 0-7) for the post-tx specimens (P=0.003 and <0.001, respectively).

Conclusions: As determined by immunohistochemistry, there was a significant decrease in the levels of ER and PR expression following neoadjuvant therapy with EXM in combination with celecoxib for postmenopausal women with BC. Further investigation is necessary to understand the biologic mechanism for this decreased hormone receptor expression of tumor cells, and to determine possible long-term therapeutic implications.

109 Expression of a Novel Cell-Cycle Regulated Protein HTF9C, Identifies a Subgroup of HER2 Positive Breast Cancer with a More Aggressive Clinical Course

DC Dim, DG Hicks, BJ Yoder, RR Tubbs, TG Budd, BZ Ring, RA Beck, NC Estopinal, RS Seitz, DT Ross. Roswell Park Cancer Institute, Buffalo, NY; MicroPath Labs, Lakeland, FL; Cleveland Clinic Foundation, Cleveland, OH; Applied Genomic Inc, Burlingame, CA; Comprehensive Institute of Huntsville, Huntsville, AL.

Background: Over-expression of HER2 in a subset of breast cancers (HER2+) is associated with higher grade tumors that are clinically more aggressive. Despite these distinctive clinical features, significant heterogeneity exists among patients with HER2+ disease with roughly half of these tumors expressing hormone receptors, as well as demonstrating varying degrees of responsiveness to Trastuzumab therapy. The identification of factors that may help better define the clinical diversity of HER2+ tumors would likely benefit clinical decision-making and suggest new approaches to overcome Trastuzumab resistance.

Design: Tissue microarrays from two established breast cancer cohorts (CCIH & CCF, J Clin Oncol 24:3039-47,2006) were used to investigate potential IHC markers to help stratify HER2+ breast cancer patients into different prognostic categories. 37 Antibodies were selected based on their ability to reproducibly divide the HER2+ patients into two independent subgroups. The CCIH cohort was used to identify the subset of markers with a univariate association of IHC staining with clinical outcome and these associations were tested on the CCF cohort.

Results: An antibody to HTF9C showed strong correlation with likelihood of recurrence at 5 years within 67 HER2+ patients from the CCIH cohort (HR 4.80; 95% CI 1.77 to 13.10, $p < 0.002$). The sensitivity and specificity were 50% & 87%. This prognostic

correlation was validated on 75 HER2+ patients from the CCF cohort (HR 3.63; 95% CI 1.26 to 10.50, p < 0.02). The sensitivity and specificity were 60% & 70%. When the two cohorts are combined *in silico*, the sensitivity and specificity were 55% & 79%.
Conclusions: HTF9C, a novel, cell-cycle regulated, protein is differentially expressed across tumors (Mol Biol Cell 13:1977-2000,2002) and correlates with poor prognosis in ER+ positive breast cancer and lung cancer. Here we show that in two independent cohorts, it correlates with poor prognosis in HER2+ patients. As these patients were not treated with Trastuzumab before the recurrence event, it is of great interest to see how HTF9C correlates with prognosis in a HER2+ population treated adjuvantly with Trastuzumab.

110 Ductal Carcinoma In Situ (DCIS) with Regressive Changes: Recognition, Immunoprofile and Biologic Behavior

AB Domfeh, M Chivukula, G Carter, D Dabbs. Magee Womens Hospital, UPMC, Pittsburgh, PA.
Background: Regressive changes (RC) described in Malignant Melanomas (MM) signifies some degree of host response to tumor and poor prognosis.We have observed similar RC in a subset of high grade DCIS in our breast specimens. The significance of DCIS with regressive changes (DCIS-RC) is not described so far. The aim of our study is understand the biologic behavior of RC.
Design: We defined DCIS-RC as presence of peridutcal fibrosis, dense lymphocytic infiltrate and thin rim of intact epithelium linng ducts. 38 cases of DCIS-RC and 21 cases of DCIS, nuclear grade 3 were retrieved from our departmental archives. A panel of immunomarkers were selected : Basal phenotype markers (Cytokeratins 5/6, 14, 17 and EGFR); Intercellular adhesion molecule-1(ICAM-1), a marker of early metastasis and poor prognosis; Bcl-2, a marker of progression of disease; C-met a receptor for Hepatic growth factor (HGF), which is associated with increased tumor invasion, migration and angiogenesis. The cytokeratins were graded as negative or positive. EGFR is graded similar to Her2/neu (0, 1+, 2+, and 3+). ICAM and Bcl-2 were graded as negative or positive, and semi-quantified. C-met immunostaining was scored based on an intensity scoring (0-10).
Results: Please refer to Table 1 Follow-up of these cases with reference to lymph node metastasis are ongoing.
Conclusions: 1. Bcl-2 is expressed at a higher proportion in cases of DCIS (85.7%) than DCIS-RC (44.7%), suggesting this subset may have a higher rate of progression of disease.2. C-met expression in all cases of DCIS (with or without RC) suggests an increased potential to early metastasize in this subset of high nuclear grade DCIS versus low grade DCIS. 3. ICAM expression was almost equal in both DCIS-RC (60%) and DCIS (71%) implying a majority of these cases may carry a poor prognosis.4. A great majority of DCIS-RC and DCIS express basal phenotype markers.

Immunohistochemical staining pattern of DCIS-RC and DCIS

	CK5/6	CK14	CK17	EGFR	ICAM	BCL-2	C-MET
DCIS-RC	25/38 (65.8%)	26/38 (68.4%)	30/38 (78.9%)	22/38 (57.9%)	23/38 (60.5%)	17/38 (44.7%)	38/38 (100%)
DCIS	21/21 (100%)	21/21 (100%)	21/21 (100%)	7/21 (33.3%)	15/21 (71.4%)	18/21 (85.7%)	21/21 (100%)

111 Predictors of Pathologic Response in Breast Cancer

EJ Duncavage, J Pfeifer, MA Watson, RL Aft. Washington University, St. Louis, MO; Washington University, Saint Louis, MO.
Background: Breast carcinoma is the most common form of cancer in women. However, methods to accurately predict disease response to chemotherapy are lacking. Using Affymetrics™ expression microarrays, we discovered a set of genes that prospectively predicts pathologic response to an Epirubicin and Taxotere chemotherapy regimen.
Design: Fresh frozen and paraffin-embedded breast biopsy tissue was collected prospectively as part of an ongoing study. The patient group included 40 ductal carcinomas, 7 lobular carcinomas, 6 mammary carcinomas, and 1 inflammatory carcinoma. At presentation, 3 patients had stage I disease, 24 stage II, 26 stage III, and 1 stage IV. Patients were then treated with a standard chemotherapy regimen including Epirubicin and Taxotere. Post- treatment mammographic data were collected to determine treatment response. Shrinkage of tumors to less than 1 cm was considered a positive pathologic response. Gene expression data were obtained using Affymetrix™ expression microarrays. The resulting data were analyzed using the Spotfire™ software package. Patients were divided into positive and negative pathologic responders and the corresponding expression data were analyzed for differences in expression using ANOVA statistics. Transcripts with expression differences at a p-value of <0.05 were considered significant. ESTs and transcripts without annotations were ignored.
Results: A subset of 340 genes with significant differences in expression between pathologic responders and non-responders was defined. Genes with the greatest differential expression were MRPL39, TTC7A, SMC1L1, RGS9, NAGA, PTPRN2, MKRN2, and LIPE—genes associated with structural maintenance and metabolism. The set also contained genes previously implicated in carcinogenesis, including retinoic acid receptor alpha (RARA), checkpoint with forkhead and ring finger domain (CHFR), BAX, MLH1, and others.
Conclusions: These data suggest that breast cancer patients can be prospectively classified as responders or non-responders to Epirubicin and Taxotere chemotherapy using gene expression data. Experiments are in progress to validate the gene set by employing immunohistochemical techniques on both matched patient samples and a second set of paraffin-embedded breast tumors using genes with commercially available antibodies (24 of 340). Additional patients have been enrolled in the study and new expression data, when available, will be added to enhance sensitivity.

112 PI3 Kinase Mutations Occur Early in Breast Carcinoma

JB Dunlap, C Le, J Patterson, A Town, MC Heinrich, CL Corless, ML Troxell. OHSU, Portland, OR.
Background: Mutational activation of protein kinases in breast carcinoma is important in defining targets for novel therapeutics. BRAF, a member of the RAF family, has been studied in small series, yielding no mutations. In contrast, mutations in the catalytic subunit of phosphoinositol-3-kinase (PI3KCA) have been described in 18-40% of invasive carcinomas, and 2 of 15 DCIS cases. PI3KCA mutations in breast carcinomas are clustered in 'hotspots' in exons 7, 9, and 20, some with transforming potential in vitro. We screened for PIK3CA and BRAF mutations in invasive carcioma, and determined whether these mutations are also present in the accompanying in-situ element.
Design: 53 cases of invasive breast carcinoma with an intermixed in-situ component were selected from the archives of OHSU. Invasive tumor was macro-dissected from formalin-fixed paraffin embedded tissue, DNA was extracted, and BRAF exons 11, 15 and PI3KCA exons 7, 9, 20 were amplified by PCR. Amplicons were screened by a Transgenomic WAVE HPLC system; suspected mutations were confirmed by bidirectional sequencing. Laser capture microdissection (LCM, PIXCELL II, Arcturus) was performed on mutation-positive carcinomas to obtain separate populations of invasive and in-situ tumor cells. The resulting DNA samples were then analyzed by nested PCR and direct DNA sequencing.
Results: Among 38 cases analyzed for BRAF there were no mutations detected in exon 11 or 15. 53 cases of invasive carcinoma were informative for PI3KCA; there were 6 exon 20 mutations (5 H1047R, 1 H1047L), 4 exon 9 mutations (3 E545K, 1 E542K), and no exon 7 mutations, (total of 10/53, 18.6%). Of 4 cases with exon 20 mutations and informative PCR from paired LCM invasive and in-situ tumor, all 4 showed identical exon 20 mutations in the in-situ tumor. Of the exon 9 cases, 1 had an exon 9 mutation from LCM in situ component matching the corresponding macrodissected invasive carcinoma; while 1 was wild type in the LCM dissected in situ component.
Conclusions: Our results substantiate the observation that BRAF mutations are unlikely to play a role in breast carcinoma. In contrast, 18.6% of our invasive carcinomas demonstrated PI3KCA mutations. Moreover, our initial studies indicate that there is concordance for PI3KCA mutations in matched samples of invasive and in-situ tumor, suggesting that PI3KCA mutations occur early in breast cancer development. This has therapeutic implications in regard to specific inhibitors in clinical development for the PI3 kinase/AKT pathway.

113 Peritumoral Lymphatic Vascular Density as a Prognostic Marker in Invasive Breast Carcinoma

YM El-Gohary, RS Saad, MJ Robinson, RJ Poppiti. Mount Sinai Medical Center, Miami Beach, FL.
Background: Lymphatic invasion and nodal metastases have a crucial role in the spread of breast carcinoma and serve as a major prognostic indicator for disease progression and a guide for therapeutic strategies. There is limited data evaluating the significance of lymphatic microvessel density (LMD) as a prognostic marker in patients with invasive breast carcinoma. In this study, we investigated tumor lymphatic as well as blood vascular densities as predictive markers for the risk of lymph node (LN) metastases and its relation to other prognositc parameters in breast cancer patients.
Design: Forty eight cases of invasive breast carcinoma (mostly ductal) treated with mastectomy and lymph node dissection were reviewed. All cases were immunostained with D2-40 and CD31. Positively stained microvessels (MV) were counted in densely lymphatic/vascular foci (hot spots) at 400x (=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. Spearman correlation was used to compare MV count with LN status, tumor size, nuclear grade, estrogen and progesterone receptors, Her2/neu, presence of angiolymphatic invasion, and clinical stage.
Results: Peritumoral LMD was significantly higher than intratumoral LMD (9 ± 7 vs 4 ± 6, P< 0.01), and correlated with CD31 MV (r=0.4, P<0.05). There was a positive correlation of both D2-40 and CD31 MV count with LN metastases (r=0.5, and 0.38), nuclear grade (r=0.36 and 0.3), and clinical stage (r=0.42 and 0.49). Only D2-40 LMD correlated significantly with the presence of angiolymphatic invasion (detected by D2-40), tumor size, histologic grade, and Her2/neu status (r=0.54, 0.34, 0.3, and 0.3 respectively). Angiolymphatic invasion was detected in 18/48 (37.5%) patients by D2-40, 11/48 (22.9%) by CD31, and 5/48 (10.4%) by routine H and E. Angiolymphatic invasion detected by D2-40 showed significant correlation with LN status (r=0.56), tumor size (r=0.35), and nuclear grade (r=0.41).
Conclusions: Our study showed that both lymphatic and blood vascular densities play an important role in the progression of breast carcinoma. Peritumoral LMD detected by D2-40 showed prognostic significance with positive correlation with LN metastases, nuclear grade, clinical stage, angiolymphatic invasion, tumor size, histologic grade, and Her2/neu status. In addition, D2-40 detected more lymphatic invasion than conventional H and E and the commonly used pan-endothelial marker, CD31.

114 Global Histone Modifications in Breast Cancer and Their Prognostic Significance

SE El-Sheikh, AR Green, EA Rakha, EC Paish, DM Heery, IO Ellis. Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom; University of Nottingham, Nottingham, United Kingdom.
Background: Global changes in histone modification show association with patient outcome in prostate cancer. However, the clinical significance of these modifications in breast cancer is unknown.
Design: Global histone modification in a well-characterised series of breast carcinomas (n=880) with long term follow-up was assessed using immunohistochemistry and tissue microarray. Specific antibodies were used to detect acetylation of H3 (Lys9 and Lys18) and H4 (Lys12), and dimethylation of histone H4 (Arg3) and H3 (Lys4). The presence of these chromatin 'marks' was correlated with clinicopathological variables and patients' outcome.

Results: Reduced levels of histone acetylation/dimethylation were observed in medullary-like carcinomas, whereas they were readily detected in normal breast tissue, lobular and tubular carcinomas. Reduced global histone acetylation/dimethylation was significantly associated with established poor prognostic variables; larger tumour size, higher stage, recurrence, distant metastases and higher mortality rate. Survival analyses showed that low detection of the histone modifications was associated with shorter overall survival and shorter disease free interval.
Conclusions: Our results show, for the first time, that global changes in specific histone modifications patterns may play an important role in breast cancer development and progression and their reduced expression is associated with poor prognosis and shorter survival.

115 Tertiary Center Breast Cancer Pathology Review: Positive Impact on Patient Care

LJ Elavathil, M Goulbourne, A Zaidi, N Howatt, K Ceballos, OB Tadross, G Gohla, T Aziz, B Strang, SK Dhesy-Thind, A Lytwyn. Juravinski Cancer Center, Hamilton, ON, Canada; McMaster University, Hamilton, ON, Canada.
Background: In many cancer clinics, the original surgical pathology material of referred-in patients undergoes consultation review. Previous studies have reported that second pathology review of breast cancer cases resulted in changes in diagnoses. We assessed whether pathology reviews of breast cancer cases led not only to revisions in diagnoses, but also whether these revisions altered subsequent recommendations for chemotherapy, radiation treatment, or additional surgical procedures.
Design: We prospectively reviewed the surgical pathology material from patients with breast cancer referred to the Juravinski Cancer Centre from February 2005 to April 2005. Each case was reviewed by 1 of 6 consultant pathologists who are members of the breast site team. The consultant pathologist reported the pathology findings using a standardized synoptic report form. Disagreements in any reported item were reviewed and confirmed either by a second consultant pathologist or by the pathology breast site team; all were masked to the opinions of the original and first consultant pathologists. The case was then presented in multidisciplinary breast cancer rounds. If the change in diagnosis altered management recommendation it was identified as a major discrepancy. All others were recorded as a minor discrepancy.
Results: We reviewed the pathology reports and slides from 133 patients. Post-review diagnoses were: 105 (79%) invasive breast cancers with or without in situ disease, 20 (15%) in situ carcinoma alone, 1 (0.8%) metastatic breast carcinoma, 1 (0.8%) non-breast metastatic carcinoma, and 6 (5%) benign breast changes. Forty-three (32%) women had axillary lymph node involvement; 11 (8%) women had more than 4 nodes involved. Seventy-nine discrepant items were identified in 61 (46%) cases: 1, 2 and 3 discrepant items were noted in 44, 15, and 2 cases, respectively. Twenty-one of the 79 (27%) discrepant items were related to tumor size, 20 (25%) to margin status, and 9 (11%) to tumor grade. Eighteen reports (14%) contained a major discrepancy, and resulted in changes in chemotherapy recommendations for 5 (4%) patients, radiation for 9 (7%) patients and additional surgery for 4 (3%) patients.
Conclusions: Consultant pathology review resulted in major changes in therapeutic recommendations for a substantial number of patients diagnosed with breast cancer.

116 *FGFR1* Amplification in Breast Carcinomas: A Chromogenic *In Situ* Hybridisation Analysis

SE Elsheikh, A Green, MB Lambros, D Powe, IO Ellis, JS Reis-Filho. University of Nottingham, Nottingham, United Kingdom; Institute of Cancer Research, London, United Kingdom.
Background: The amplicon on 8p11.2 is reported to be found in up to 15% of breast carcinomas. It has recently been demonstrated that this amplicon has 4 separate cores. The second core encompasses important oncogenes candidates, including the Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) and fibroblast growth factor receptor 1 (*FGFR1*) genes. *FGFR1* encodes a transmembrane tyrosine kinase receptor that has recently been therapeuticically targeted in haematological malignancies. Recent studies have demonstrated that specific *FGFR1* amplification correlates with gene expression and that FGFR1 activity is required for the survival of MDA-MB-134 breast cancer cells.
Design: *FGFR1* gene amplification was analysed in tissue microarrays (TMAs) comprising a cohort of 880 unselected breast tumours by means of chromogenic in situ hybridisation (CISH). In-house generated probes mapping to the second core of the 8p11.2 amplicon (*FGFR1*) were used. CISH signals were counted in a minimum 60 morphologically unequivocal neoplastic cells. Amplification was defined as >5 signals per nucleus in more than 50% of cancer cells, or when large gene copy clusters were seen.
Results: After excluding non-informative cores, results for 496 cases were available. *FGFR1* amplification was observed in 8.7% of the tumours and was significantly more prevalent in patients >50 years of age. *FGFR1* amplification showed an inverse correlation with progesterone and androgen receptor expression (all, $p<0.05$) and a trend for high histological grade, more advanced stage, poor Nottingham prognostic index, presence of vascular invasion and lack of basal-like phenotype (all, $p<0.1$). Univariate analysis revealed a significant association between *FGFR1* amplification and reduced overall survival (OS) ($p<0.01$) and a marginal association on Cox Hazard multivariate analysis ($p<0.1$).
Conclusions: Given that up to 8.7% of all breast cancers harbour *FGFR1* amplification and that this amplification is associated with markers of aggressiveness and a shorter OS, further studies analysing the *FGFR1* as a potential therapeutic target for breast cancer patients are warranted.

117 Columnar Cell Lesions in Breast Core Needle Biopsy and the Predictive Value for Unsampled Ductal Carcinoma

J Eradat, JM Shamonki, LW Bassett, S Apple. University of California, San Diego, San Diego, CA; University of California, Los Angeles, Los Angeles, CA.
Background: Columnar cell lesions (CCL), frequently associated with microcalcifications, have been increasingly sampled by core needle biopsy (CNB) partly due to improved detection by digital mammography. Recent studies implicate CCLs as a marker for an increased risk of breast cancer, and recommend excisional biopsy for a finding of CCL with atypia on CNB. This study aims to identify the risk for adjacent unsampled ductal carcinoma when CCLs are identified in a CNB specimen.
Design: We retrospectively reviewed 76 ultrasound-guided CNB specimens showing CCLs performed for mammographic calcifications between 2003 and 2006 at our medical center. Biopsies containing atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) or papillary lesions were excluded. The remaining 57 specimens were divided into columnar cell change (CCC) or hyperplasia (CCH) lesions and further described as with or without atypia. 13 patients underwent subsequent excisional biopsy, the results of which were reviewed for the presence of significant pathologic findings including ADH, DCIS or invasive ductal carcinoma.
Results: Microcalcifications were demonstrated in all biopsies, corresponding to the mammographic findings. Of 17 CCC lesions identified, all lacked atypia. Of these patients, 3 underwent excisional biopsy revealing no further pathologic findings. 25 cases showed CCH without atypia, and 5 underwent excisional biopsy, revealing 1 case of previously unsampled ADH (20%). 15 CCH lesions showed atypia on CNB, 5 of which underwent subsequent excisional biopsy. Of those 5 cases, previously unsampled ADH and DCIS were present in 2 (40%) and 1 (20%), respectively, of the excisional biopsy specimens. No invasive carcinoma was identified. Incidentally noted LCIS was identified in 2 excisions performed for CCL without atypia.
Conclusions: In our population, the presence of CCH with atypia in the CNB confers a 20% risk for unsampled DCIS in the surrounding breast tissue. Although the mammographic calcifications were sampled by the CNB, an adjacent carcinoma is not ruled out when atypical CCH is identified. This study supports the recommendation that CCH with atypia should be followed by excisional biopsy. No excision is necessary for CCC without atypia unless a mammographic mass lesion is not accounted for by the CNB diagnosis.

118 Basal-Like Subtype Breast Cancers in Women Older Than 40 Years of Age

CH Ersahin, M Chivukula, R Bhargava, DJ Dabbs. Magee-Women's Hospital of UPMC, Pittsburgh, PA.
Background: *Basal-like* carcinoma is one of the five distinct subtypes of breast tumors that have been identified by gene microarray technologies. These carcinomas are distinguished by the expression of keratins that are characteristic of myoepithelial cells, hence the term *basal-like*. There is a significant overlap between BRCA-1 associated cancers and *basal-like* carcinomas since BRCA-1 tumors have an expression profile consistent with a basal phenotype. *Basal-like* cancers have been well described in women younger than 40 years of age. However, there is little data about this carcinoma in patients older than 40.
Design: A total of 17 breast cancers from patients over 40 years of age, which showed less than 10% staining for ER, PR and negative HER2 (triple negative) between 2002-2006 were evaluated. Criteria for the diagnosis of *basal-like* carcinoma included positivity for basal keratins (CK 5/6, 14, 17) and EGFR. Morphologic criteria included tumor circumscription, lymphoid infiltrates, Nottingham score 8-9, and +/- necrosis. Two out of 4 positive immunohistochemical positivity along with a triple negative ER, PR, and Her2 status was considered as consistent with basal phenotype.
Results: Fourteen of the 17 breast cancer specimens (mean age: 55.7 and range: 41-68) expressed at least 2 of the basal keratins and EGFR. Morphologically, all tumors had high nuclear grade and high mitotic activity. Thirteen cases had syncytial growth pattern, while one case had ductal pattern. Although some cases had pushing margins, most had infiltrative margins with lymph node metastasis. Lymphoplasmacytic infiltration was not a consistent finding. Eight cases had no DCIS component, eight had approximately 5% DCIS component and the remaining case had 50% DCIS with associated comedo necrosis.
Conclusions: Our study demonstrates that there is a subset of patients, > 40 years of age who have morphologic and immunohistologic features of the *basal-like* breast carcinoma. The morphologic and immunohistologic findings are identical to those that are seen in patients < 40years of age who have early-onset breast carcinoma associated with BRCA-1 mutation. These patients require further investigation for BRCA-1 analysis, as well as gene-microarray analysis to compare the gene expressions with those seen in the younger population.

119 Histomorphologic Analysis of 143 Papillary Lesions on Core Needle Biopsy of the Breast with Follow-Up Excision

NN Esposito, RR Johnson, GM Ahrendt, MA Gannott, S Quaynor, A Soran, DJ Dabbs. Magee-Womens Hospital of UPMC, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA.
Background: Core needle biopsies (CNB) of papillary lesions of the breast are currently managed by excision due to the risk of higher-grade lesions within the papilloma or in adjacent tissue (synchronous) on follow-up excision (FUE). The aim of this study was to determine whether there are predictive features of atypia or malignancy on pathologic exam of these lesions on breast CNB.
Design: Papillary lesions diagnosed by breast CNB between 2001-05 were analyzed after excluding CNB with synchronous atypia and/or carcinoma and those without FUE. Histologic features and the presence of adjacent pathology on CNB and resection specimens were analyzed. Logistic regression analysis was performed to test for statistical significance.

Results: 143/286 CNB of papillary lesions had FUE available for analysis: 111 (78%) benign papillomas, 18 (13%) atypical papillomas, 12 (8%) papillary lesion NOS, and 2 (1%) papillary carcinomas. 26% of benign papillomas were upstaged to atypical or malignant and 50% of atypical papillomas were upstaged to malignant on excision. 3 risk groups were extrapolated from those with benign papillomas as follows, with percents representing frequency of a higher-grade lesion on FUE: central papilloma with no mitoses (9%), peripheral papilloma with no mitoses (23%), and central or peripheral papilloma with mitoses (38%). After stratifying data by diagnosis on CNB, only myoepithelial cell absence (p=.014, 96% specificity) and cytologic atypia (p=.036, 99% specificity) predicted higher-grade lesions on FUE of benign papillomas, though the sensitivity of either was <50%.

	HIGHEST-GRADE DIAGNOSIS ON FOLLOW-UP EXCISION						
CNB DIAGNOSIS	Papilloma or FCC	Atypical papilloma	ADH/ ALH, sync	DCIS/LCIS, within papilloma	DCIS/ LCIS, sync	Papillary carcinoma	Invasive carcinoma, sync
Papilloma	74%	5%	14%	3%	4%	-	-
Atypical papilloma	11%	33%	6%	17%	11%	17%	6%
Papillary lesion NOS	50%	25%	8%	-	-	8%	8%

FCC=fibrocystic changes, sync=synchronous diagnoses in tissue adjacent to papilloma;NOS=not otherwise specified

Conclusions: Though patients may be stratified into risk groups based on papilloma location and mitotic activity, there are no histopathologic features of papillary lesions on breast CNB that predict pathology on subsequent excisions with high sensitivity.

120 Diagnostic Value of Telomerase Expression in Breast Fine Needle Aspiration Biopsies

G Fischer, O Tutuncuoglu, M Bakhshandeh, S Masood. University of Florida HSC, Jacksonville, FL.

Background: Fine needle aspiration biopsy (FNAB) of breast is a minimally invasive procedure for evaluation of patients with palpable breast lesions. It is a cost effective and relatively non-traumatic procedure that has replaced surgical biopsy in most of institutions. There are however limitations in the ability of FNAB to reliably diagnose small percentage of cases that are difficult to diagnose by cytomorphology alone. This retrospective study was designed to assess the added value of telomerase immunostain in interpretation of breast FNABs. This enzyme has been shown to be activated in different malignant tumors, including breast cancer. The detection of this molecular marker on cytologic smears and cellblocks may be helpful for interpretation of FNAB specimens.

Design: 50 benign and 50 malignant breast lesions were retrieved from our archive files (Dept.Pathology University of Florida/Shands). These cases had an initial FNAB and a follow-up excisional biopsy, lumpectomy or mastectomy. The cases were assigned to the benign or malignant groups based on the histologic diagnosis of the surgical specimen. The Papanicolaou-stained smear and cell blocks of FNABs were immunostained to detect telomerase activity. The expression of telomerase was detected as dot-like nuclear staining pattern and results were compared to morphologic diagnosis.

Results: In our study, positive telomerase immunostaining was observed in 56 % (28/50) of malignant and only 4% (2/50) of benign breast lesions. This difference was statistically significant (p= 0.00001). The telomerase immunostain showed remarkably high specificity (96%) and positive predictive value (93%) accompanied by relatively low sensitivity (56%) and negative predictive value (69%).

Conclusions: Our study shows the potential value of telomerase immunostain as a diagnostic adjunct in challenging breast fine needle aspirates. The high specificity and high positive predictive value of this test makes it easier to render of a more definitive diagnosis. Expression of telomerase on highly suspicious breast fine needle aspirations may upgrade the diagnosis to malignancy. This approach will assist in reducing the number of unnecessary follow-up diagnostic surgical biopsies. This information has to be integrated into combination of clinical presentation and breast imaging as a triple test in order to eliminate under/overdiagnosis of malignancy.

121 EGFR Gene Amplification Is an Uncommon Event in Basal-Like Breast Carcinomas

M Flanagan, U Surti, M Chivukula, GJ Carter, DJ Dabbs, R Bhargava. Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: Basal-like breast carcinoma is a recently recognized entity. These tumors are characteristically "triple negative" and express basal type cytokeratins. They are more commonly encountered in younger patients with *BRCA1* germ-line mutation and so far appear to have an aggressive course. These tumors also express EGFR by immunohistochemistry (IHC), but have not been extensively studied for *EGFR* gene amplification or *EGFR* hotspot mutations.

Design: Seventeen basal-like breast carcinomas, showing characteristic morphologic and immunophenotypic features (high grade, geographic necrosis, host lymphocytic response, and good circumscription, negativity for ER, PR and HER2, and immunoreactivity for basal-like cytokeratins) were studied for *EGFR* gene by fluorescent in situ hybridization (FISH) and EGFR protein by IHC. A tissue microarray with 3-fold redundancy was constructed from these 17 cases. Four microns TMA sections were subjected to dual color FISH and IHC staining for EGFR. Number of EGFR copies and ratio of EGFR gene to chromosome 7 signals was recorded, and a ratio of 2 or higher was considered amplified. EGFR-IHC stains were scored from 0 to 3+ using criteria similar to Herceptest®.

Results: All 17 cases were negative for *EGFR* gene amplification. Thirteen cases showed normal *EGFR* gene copy number. Four cases showed polyploidy/aneuploidy (4 copies for both *EGFR* gene and chromosome 7). There was no correlation between increased gene copy number and IHC staining (table 1). There was no correlation between EGFR IHC staining with respect to tumor size and lymph node status (table 2).

Table 1

	EGFR-IHC 0	EGFR-IHC 1+	EGFR-IHC 2+	EGFR-IHC 3+	Total
Normal EGFR gene copies	2	4	7	0	13
Polyploid (4 copies for both EGFR and chromosome 7)	0	1	2	1	4
Total	2	5	9	1	17

Table 2

	Average Tumor Size	Lymph Node Status
EGFR 0 and 1+ (n=7)	2.17 cm	1 case with isolated tumor cells and 1 case with 9 positive lymph nodes; 5 other negative
EGFR 2+ and 3+ (n=10)	1.76 cm	2 cases with micrometastasis; 8 other negative

Conclusions: A 3+ EGFR IHC staining is uncommon in basal-like carcinoma. Although most basal-like carcinoma shows some degree of EGFR staining by IHC (usually 1+ and 2+), it is often unrelated to *EGFR* gene amplification. Whether EGFR expression is clinically significant, or blocking protein expression would be clinically useful, would require randomized clinical trials to answer these questions.

122 Are the Different Molecularly-Defined Subtypes of Invasive Breast Cancer Associated with Different Reproductive Risk Factors?

LS Galaburda, RM Tamimi, HJ Baer, AC Deitz, GA Colditz, SJ Schnitt, LC Collins. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; Brigham and Women's Hospital and Harvard Medical School; Harvard School of Public Health; Boston, MA; GlaxoSmithKline, Collegeville, PA.

Background: Prior epidemiologic studies have established that there is an association between various reproductive factors such as age at menarche, age at first birth, age at menopause and parity and the risk of developing breast cancer. However, breast cancer is a phenotypically heterogeneous disease, and it is possible that the importance of these established reproductive risk factors may differ according to the breast cancer phenotype.

Design: We constructed tissue microarrays (TMAs) by obtaining triplicate 0.6mm cores from paraffin blocks of 3167 breast cancers that developed in women enrolled in the Nurses' Health Study over twenty years of follow-up (1976-1996). Sections cut from the TMAs were immunostained with a variety of biomarkers, including ER, PR and HER2. To date, ER, PR and HER2 immunostains have been analyzed in 872 invasive cancers in this population. Results of these immunostains were used as a surrogate to classify the cancers into four molecular subtypes as previously defined by expression array analysis: 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/triple negative (ER-, PR-, HER2-). Reproductive risk factors including age at menarche, age at first birth, age at menopause and parity were compared among these four groups.

Results: Among the 872 breast cancers studied to date, 71% were luminal A, 4% luminal B, 6% HER2, and 19% basal-like/triple negative types. Age at menarche, age at first birth, and age at menopause were similar across the four breast cancer categories. However, women with luminal B and HER2 types were significantly more likely to be nulliparous (15.3% and 11.7%, respectively) than those with luminal A and basal-like/triple negative types (6.4% and 5.1%, respectively)(p=0.04).

Conclusions: The results of this study suggest that the impact of reproductive factors on breast cancer risk may differ according to the molecularly-defined breast cancer type. In particular, these results raise the possibility that nulliparity may be preferentially related to the development of HER2+ breast cancers.

123 Comparison of the Prevalence of Molecular Phenotypes in DCIS and Invasive Breast Cancer: A Tissue Microarray-Based Analysis of 977 Cases

MA Galan, RM Tamimi, HJ Baer, AC Deitz, GA Colditz, SJ Schnitt, LC Collins. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston; Brigham and Women's Hospital and Harvard Medical School; Harvard School of Public Health; Harvard Center for Cancer Prevention, Boston, MA; GlaxoSmithKline, Collegeville, PA.

Background: Studies using DNA microarrays have identified four distinct categories of invasive breast cancer: luminal A, luminal B, HER2 and basal-like. Immunostaining for ER, PR and HER2 can be used to help identify these subtypes. In this study, we compared the prevalence of these molecular subtypes among cases of DCIS and invasive breast cancer using ER, PR, and HER2 immunophenotyping as a surrogate for gene expression analysis.

Design: Tissue microarrays (TMAs) were constructed from paraffin blocks of 3167 breast cancers that developed in women enrolled in the Nurses' Health Study over a 20 year period (1976-1996). TMA sections were immunostained for ER, PR and HER2. To date, ER, PR and HER2 immunostains have been analyzed in 977 of these cases (105 DCIS and 872 invasive cancers). Using the ER, PR and HER2 immunostain results on the TMA sections, DCIS and invasive cancer were each grouped into one of four categories: ER+ and/or PR+ and HER2- (luminal A); ER+ and/or PR+ and HER2+ (luminal B); ER- and PR- and HER2+ (HER2); ER-, PR-, HER2- (basal-like).

Results: The four molecularly-defined categories previously identified among invasive breast cancers were found among the DCIS cases. The frequency of these categories among DCIS cases and invasive cancers are compared in the table:

Molecular Category	DCIS (n=105)	Invasive (n=872)	p-value
Luminal A (ER+ and/or PR+ and HER2-)	60.0%	71.1%	0.02
Luminal B (ER+ and/or PR+ and HER2+)	12.4%	3.9%	0.0001
HER2 (ER-, PR-, HER2+)	13.3%	6.0%	0.004
Triple Neg/Basal-like (ER-, PR-, HER2-)	14.3%	19.0%	0.25

Tumors with the luminal A phenotype were significantly more frequent among invasive breast cancers, whereas luminal B and HER2 types were significantly more frequent among DCIS cases.

Conclusions: The prevalence of the luminal A, luminal B and HER2 phenotypes differed significantly between DCIS and invasive breast cancers. Luminal A lesions were more common among invasive cancers and luminal B and HER2 types more common among DCIS. This difference may have important implications regarding breast cancer progression.

124 Assessment of Histologic Features and Biomarkers of Breast Carcinoma Associated with Pathologic Complete Response to Neoadjuvant Chemotherapy

X Gao, Q Khan, M Davis, C Connor, OW Tawfik, PA Thomas, F Fan. KU Medical Center, KC, KS.

Background: Neoadjuvant chemotherapy (NACT) represents the standard of care in locally advanced breast cancers. NACT increases the rate of breast preservation and provides information on the primary tumor's response to chemotherapy. Pathological complete response (pCR) in breast and axilla is an excellent surrogate marker of improved disease free and overall survival and is therefore one of the goals of NACT. Only 14-27% of women will achieve pCR with chemotherapy and biomarkers that may predict a response to chemotherapy are needed. The aim of this study was to assess histologic features and biomarkers in the pre-treatment biopsy specimen to identify predictive factors for pCR after NACT. Such information may prove useful in selecting patients for NACT.

Design: Information on 50 women treated with NACT after an initial diagnosis of primary invasive breast carcinoma was obtained retrospectively from our database and chart review at KU Medical Cancer. Lumpectomy or mastectomy was performed after NACT. The pathologic response in the surgical excision specimen was divided into complete response (pCR, no residual invasive tumor identified), partial response (PR, small foci of residual tumor and treatment effects identified), and no response (NR, no change in tumor size with no treatment effect identified). Histologic features (tumor size, nuclear grade, histologic type and histologic grade) and biomarkers (ER, PR, and Ki-67) in the pre-treatment biopsy specimens were correlated with the corresponding pathologic response in the subsequent surgical excision specimens.

Results: Among 50 women, 11 achieved pCR, 19 had PR and 20 had NR. Original tumor size, nuclear grade, histologic grade did not correlate with a pathologic response after NACT. All 11 women with pCR were ER-/PR- and had high Ki-67 (>30%) in their original tumors before NACT.

	N	ER - (<10%)	PR - (<10%)	Ki-67 (>30%)
Complete response	11	11/11	11/11	11/11
Partial response	19	10/19	8/19	11/19
No response	20	4/20	7/20	2/20

Conclusions: Tumor size, nuclear grade and histologic grade in the primary breast cancer did not have significant predictive value for pathologic response to NACT in breast carcinomas. However, negative ER/PR combined with high Ki-67 in the primary tumor predicted high rate of pathologic response to neoadjuvant chemotherapy.

125 Pure Apocrine Carcinomas of the Breast Overexpress EGFR and Have Increased EGFR Gene Dosage Due to Polysomy of Chromosome 7

Z Gatalica, L Lee, L Hatcher, J Palazzo, P Adegboyega, O Tawfik, N Bilalovic. Creighton University Medical Center, Omaha, NE; Thomas Jeferson University Hospital, Philadelphia, PA; LSU Health Sciences Center, Shreveport, LA; Kansas University Medical Center, Kansas City, KS; Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegowina.

Background: Pure apocrine carcinomas (PAC) are tumors composed entirely of epithelium showing apocrine differentiation (large cells with prominent eosinophilic, flocculent cytoplasm with sharply defined borders and large nucleus with prominent macronucleolus) along with characteristic immunophenotypic profile (androgen receptor positive, GCDFP-15 positive, estrogen receptor-alpha negative and progesterone receptor negative). Epidermal growth factor receptor (EGFR) is expressed in less than 10% of all breast carcinomas, but may be more frequently found in special types such as basal-like mammary carcinomas. Alteration of EGFR signaling plays important role in cancer development and EGFR targeted therapies may provide additional options for treatment of aggressive cancers.

Design: 25 PAC and 13 apocrine-like carcinomas (cytomorpholgically similar to PAC but without characteristic immunophenotype) were evaluated for the EGFR protein expression using immunohistochemistry (mouse monoclonal anti-EGFR antibody, Zymed Laboratories) and image analysis (Chromavision). PAC were further evaluated for *EGFR* gene and chromosome 7 copy number using fluorescent in-situ hybridization (FISH) with *EGFR/CEP7* dual color probes (Vysis).

Results: EGFR was detected in the majority (88%) of PAC. The expression was usually strong (3+) and diffuse (>50% of cells). In contrast, apocrine-like carcinomas expressed EGFR sporadically (23%). FISH showed that PAC have on average 5.5 copies of *EGFR* gene and 5.0 chromosomes 7 (*CEP7*) per nucleus (ratio 1.1). Conventional cytogenetic analysis confirmed gain of chromosomes 7 in one case of PAC (with hypotriploid chromosomal content).

Conclusions: Pure apocrine carcinomas of the breast are characterized by consistent overexpression of EGFR and increased number of *EGFR* gene copies. The underlying mechanism appears to be increased number (aneusomy) of chromosomes 7, rather than gene amplification. This information may be useful in planning adjuvant therapy for advanced apocrine carcinoma (such as tyrosine kinase inhibition).

126 Frequent Loss of TSLC1 and DAL-1 Expression in Human Breast Cancer Cell Lines and Tissues

J Geradts, G Heller, B Ziegler, I Newsham, M Filipits, E-M Markis-Ritzinger, D Kandioler, W Berger, W Stiglbauer, D Depitsch, R Pirker, C Zielinski, S Zoechbauer-Mueller. Duke University Medical Center, Durham, NC; Medical University of Vienna, Vienna, Austria; General Hospital Wiener Neustadt, Vienna, Austria; M.D. Anderson Cancer Center, Houston, TX.

Background: *TSLC1* (tumor suppressor in lung cancer 1, *IGSF4*) and *DAL-1* (differentially expressed in adenocarcinoma of the lung 1, *EPB41L3*) are tumor suppressor genes involved in cell adhesion. There is little information on the role of these genes in breast cancer.

Design: We studied eight common human breast cancer cell lines and 95 primary breast carcinomas. The expression levels of both genes were examined by reverse transcrition (RT-) PCR and by immunohistochemistry (IHC). Promoter methylation of both genes was assessed by methylation specific (MS-) PCR. Methylation status was correlated with a variety of clinico-pathologic variables.

Results: *TSLC1* expression was lost in 5 of 8 (63%) and *DAL-1* expression was lost in 6 of 8 (75%) breast cancer cell lines, respectively. Downregulation of TSLC1 expression was observed in 43 of 50 (86%), and of DAL-1 expression in 26 of 55 (47%) primary breast carcinomas. *TSLC1* methylation was found in 4 of 8 (50%) and *DAL-1* methylation was observed in 6 of 8 (75%) cell lines, respectively. Of 95 breast tumors, 46 (48%) were *TSLC1*, and 26 (27%) were *DAL-1* methylated. Twenty of 43 (47%) and 10 of 26 (38%) primary breast cancers with loss of TSLC1 or DAL-1 expression showed no methylation of the respective gene. Re-expression of *TSLC1* and *DAL-1* was observed after treatment of BT-20 cells with demethylating agents. Methylation was correlated with high tumor grade and ER/PR negativity.

Conclusions: Our findings suggest that *TSLC1* and *DAL-1* are involved in the pathogenesis of human breast cancer. Downregulation of these genes is a common event and frequently is associated with promoter methylation. However, these two genes also appear to be inactivated by other, yet to be identified mechanisms.

127 DNA Copy Number Changes Associated with Nodal Metastasis in Human Breast Carcinomas Detected by High Density Array Comparative Genomic Hybridization

J Geradts, DP Gaile, H Huang, S Liao, J Groth, D McQuaid, N Nowak, J Conroy, M Desouki. Duke University Medical Center, Durham, NC; Roswell Park Cancer Institute, Buffalo, NY.

Background: We employed high density BAC arrays interrogating the whole tumor genome, in an attempt to identify DNA copy number gains and losses that distinguish human breast carcinomas with and without the capacity to metastasize. We focused on small (T1), high grade, ER-positive invasive ductal carcinomas that are particularly heterogeneous with regard to their clinical behavior.

Design: We selected 29 tumors and their paired lymph node metastases, as well as 24 phenotypically similar tumors that had not metastasized within at least 5 years. DNA was extracted from macrodissected paraffin sections and hybridized to microarrays containing some 19,000 different BAC clones. Biostatistical algorithms were employed to determine differential copy number changes in the three groups of tumors (unmatched primaries [UP], matched primaries [MP], matched lymph node metastases [ML]), and to determine the genetic relationship between MP and ML tumors.

Results: The fractional DNA copy number losses were higher in MP (3.1%) *versus* UP (1.5%). The most common copy number gains and losses were different in the three groups of tumors. Statistical analysis revealed a number of BAC clones that distinguished UP from MP tumors, suggesting amplification or deletion of metastasis suppressing or promoting genes. The gain or loss of another set of BACs was significantly different between MP and ML tumors. Our data suggest that metastases can form at variable points in the development of a breast cancer and that subsequently the primary tumor and the metastasis independently develop additional genetic changes. Validation studies are in progress to identify the genes that may be associated with the generation and propagation of nodal metastases.

Conclusions: Breast carcinomas forming nodal metastases were characterized by a higher proportion of DNA copy number losses compared to non-metastasizing tumors, suggesting deletion of yet to be identified metastasis suppressor genes. We identified a number of gains and losses that distinguished those two groups of tumors, and a different set of DNA copy number changes differentiated primary tumors from their paired nodal metastases.

128 Immunophenotypic Profile of the Basal-Like Subtype of Invasive Breast Carcinomas: Influence on Diagnosis and Therapy

LC Goldstein, SE Tirrell, TS Barry, SJ Kussick, PL Kandalaft, PM Kim, AM Gown. PhenoPath Laboratories, PLLC and IMPRIS, Seattle, WA.

Background: Gene expression studies have identified a subset of invasive breast carcinomas with a basal-like gene expression pattern, corresponding to 15-20% of breast cancers. Patients with this breast cancer subset have a particularly poor clinical outcome. Studies using tissue microarrays have shown that these carcinomas demonstrate an immunohistochemical (IHC) profile characterized by negative expression of estrogen receptor (ER), progesterone receptor (PR) and HER2, and positive expression of cytokeratin 5/6 (CK5/6), epidermal growth factor receptor (EGFR), and c-KIT in a subset of cases. We undertook a study to expand this IHC profile on biopsy and resection specimens of invasive breast carcinomas and metastases from tumors of unknown origin in order to identify their relationship with the basal-like phenotype.

Design: A series of 102 breast carcinomas identified as negative for ER, PR, and HER2 were assessed by IHC for expression of CK5/6, EGFR, p53, p63, c-kit, mammaglobin, GCDFP-15, and MOC-31. Specimens were biopsies and resections as well as metastases. Immunostaining was performed using commercially available antibodies, and scoring was based on percentage of positive staining using conventional criteria. Additionally, a subset of tumors were tested for EGFR gene amplification by fluorescence in situ hybridization (FISH).
Results: All of the breast carcinomas were negative for ER, PR, and HER2. The IHC profile showed positivity rates of 76% for CK5/6, 66% for EGFR, 62.1% for c-kit, 53.5% for p53, and 49% for p63. Fewer cases were positive for GCDFP-15 (27.8%) and mammaglobin (18.9%) but 91.4% cases were positive for MOC-31. 1/27 carcinomas demonstrated amplification for EGFR by FISH.
Conclusions: This study expands on previously published reports showing that the basal-like subtype of infiltrating breast cancers has an immunophenotype with high positivity rates for CK5/6, EGFR, c-kit, p53, p63, and MOC-31. Clinicopathologic correlation is important in interpreting IHC markers in the context of tumors of unknown origin in that this basal subtype could be confused with carcinomas of squamous origin (positive for CK5/6, p63). The adenocarcinoma marker MOC-31 may be useful in this differential diagnosis. These tumors, positive for EGFR by IHC, may be targets of therapies such as gefitinib, but only 1/27 tumors showed gene amplification for EGFR by FISH.

129 Monomorphic Epithelial Proliferations (MEPs): A Distinctive Subtype of Epithelial Hyperplasia and Evidence Suggesting They Are the Precursor Lesion of Some Ipsilateral Breast Failure Carcinomas in Patients Treated with Breast Conserving Therapy

NS Goldstein, F Vicini. William Beaumont Hospital, Royal Oak, MI.
Background: Why some BCT-treated invasive breast carcinoma patients, including those with adequately-excised, margin-negative initial excisions develop clonally-related ipsilateral breast failures are unknown. We studied a distinctive subtype of epithelial hyperplasia around initial invasive carcinomas, which we term monomorphic epithelial proliferations (MEPs) in regards to a possible relationship with initial or IBF carcinomas.
Design: The unifying and distinctive features of MEPs were slightly-hyperdense overpopulation of highly monomorphic, clonal-like epithelial cells. 70 BCT invasive carcinomas with IBFs were retrieved from our files and the clonal relationship between the initial and IBF carcinomas was established using a 24-marker LOH PCR-assay. The histologic grade, morphology, and the amount of invasive carcinoma and DCIS, and TDLUs with MEPs near (within 5 mm) of the initial excision specimen final margin was recorded. Two BCT, non-IBF cases, matched for carcinoma grade, amount of carcinoma near the margin, and length of follow-up period for each IBF case (n=140) were selected from our files as controls.
Results: The mean number of MEPs around initial excision specimen margins in IBF patients was 5.5 compared to 3.6 in non-IBF patients ($p < 0.001$). Among the subset of patients with negative or near-least amount initial excision specimen margins, the mean number of MEPs in IBF patients was 5.7 compared to 3.3 in non-IBF patients ($p<0.001$). Patients who developed clonally related IBFs had a higher mean number of MEPs than clonally different IBF patients (6.2 vs 4.1, $p=0.056$). There was a similar trend in the subset of IBF patients with negative or near-least amount margins (6.4 vs 4.4, $p=0.061$). The mean number of MEPs near the final margin had no association with invasive carcinoma grade. In clonally related IBF cases, the mean number of identical LOHs in MEPs--initial CAs and MEPs---IBF CAs were 4.1 and 4.5, respectively, compared to mean shared numbers of 2.8 and 3.9 in clonally different IBF cases.
Conclusions: MEPs appear to be the pool of partially transformed clonal lesions from which initial invasive and IBF carcinomas arise. From this perspective, radiation therapy may work to reduce the IBF rate in most patients by eradicating these partially transformed clones and preventing new carcinomas from emerging rather than eradicating microscopic residual disease.

130 Benign Inclusions and Papillomas Presenting in Axillary Lymph Nodes

NM Granja, FI Boulos, ME Sanders, JF Simpson, DL Page. Vanderbilt University, Nashville, TN.
Background: The evaluation and reporting of axillary lymph node status is one of the most important predictors of prognosis in breast cancer. It is therefore crucial to differentiate between true metastatic lesions and other non malignant epithelial elements in the lymph node parenchyma. Lymph node inclusions are rare and have been described in different anatomic sites. The vast majority of these inclusions are epithelial and hence elicit diagnostic problems, especially in the presence of carcinoma in an extranodal site.
Design: We present a series of 8 patients with epithelial inclusions in axillary nodes, 4 of them involved by papillomas.
Results: Four patients (three females and one male) had papillomas in the axillary lymph nodes. The three female patients had 2 benign nodal papillomas, and 1 papilloma involved by ADH (atypical ductal hyperplasia). They also had concomitant breast papillomas, 2 of which were involved by DCIS and 1 by ADH. The male patient had nodal papilloma involved by DCIS and a history of breast papilloma involved by DCIS arising in gynecomastia 1 year prior. Except for areas involved by ADH/DCIS, the mammary and nodal papillomas in these patients showed defining features of benignity including bland, normally polarized epithelial cells lining fibrovascular cores, and occasional presence of a myoepithelial cell layer. The four patients with benign epithelial inclusions in the lymph nodes were all women, 3 had invasive carcinoma of the breast and one had DCIS. The inclusions were benign glands, squamous lined epithelial cyst and florid hyperplasia. Follow ups available on 2 patients with papilloma in the nodes were 14 months and 4 years and 2 months, and showed no signs of metastasis or local recurrence.
Conclusions: The presence of epithelial elements can give rise to an erroneous diagnosis of metastasis in lymph nodes with inaccurate upgrade in staging and unnecessary therapy. In addition to the fact that epithelial inclusions are significant in that they might lead to a misdiagnosis of cancer, it should also now be recognized that the glandular inclusions themselves can become neoplastic and give rise to in situ proliferative lesions like florid hyperplasia and in situ carcinoma.

131 Comparison of Oncotype-DX Recurrence Score and Standard Immunohistochemical Prognostic Markers

MC Grimes, J Coad, BJ Oliverio, KJ Bloom. CLARiENT Inc, Aliso Viejo, CA; West Virginia University, Morgantown, WV.
Background: Even with negative lymph nodes, most women with a primary breast cancer larger than 1 cm will receive chemotherapy, despite the fact that less than 20% will recur within 10 years following tamoxifen treatment. The Oncotype-DX® assay calculates a risk recurrence score (RS) based on the expression level of 16 genes assessed by RT-PCR. While the calculation is heavily weighted to genes associated with the proliferation axis, HER-2 axis and estrogen axis, it also assesses several other genes. Immunohistochemical assays are widely available to assess the protein expression of MIB-1, HER-2, ER and PR. These markers may serve as surrogates to assess the same proliferation, HER-2 and ER axes.
Design: Twenty-eight breast cancers, which were previously assessed by the OncoType-Dx® assay (Genomic Health), were retrieved from the surgical pathology files. Fourteen (48%) tumors were assessed as having a low RS; 11 (38%) an intermediate RS and 4 (14%) a high RS. Nuclear and histologic grades were blindly re-assessed from recut H&E slides. According to the manufactures' recommendations, immunohistochemical stains were performed for ER (PharmDx kit, Dako), PR,(PharmDx kit, Dako), MIB-1 (Dako), HER-2 (HercepTest kit, Dako) and P53 (D07, Dako).
Results: Eleven of the 14 (79%) low RS tumors were histologically grade 1. 13 of these 14 tumors had a MIB-1 percentage less than 10% and all of these tumors expressed PR. Of the 4 tumors with a high RS, 1 was the only tumor which over-expressed HER-2 and 1 was the only tumor that was histologic grade 3; both showed high MIB-1 expression.The remaining two high RS tumors had a MIB-1 percentage of only 1% and 2%; however, these tumors were surrounded by a lymphocytic infiltrate with high MIB-1 expression. All p53 expressing tumors (more than 5% of the tumor cells) had an intermediate RS except for 1 that had a high RS.
Conclusions: Immunohistochemical evaluation of standard IHC markers in combination with histologic grade highly correlates with low and intermediate Oncotype-Dx® RS. At least some high RS appear to be more related to an adjacent proliferative inflammatory response than the tumor itself. Since most breast cancers are currently diagnosed by core biopsy, the subsequent resection specimen sent for recurrence risk analysis can contain an inflammatory wound healing response that could then artificially increase the RS. The RS was established using tumor blocks obtained prior to the routine use of core biopsy.

132 Histopathologic Evaluation of Small Node-Negative (T1a,bN0) Invasive Breast Cancer (IBC). Prognostic Significance of Associated Ductal Carcinoma In Situ (DCIS) and Fibrocystic Changes (FCC)

M Guray, AM Gonzalez-Angulo, EO Hanrahan, K Broglio, V Valero, G Hortobagyi, AA Sahin. UT MD Anderson Cancer Center, Houston, TX.
Background: Due to improved screening methods, the majority of newly diagnosed IBCs are T1a,bN0. Although most patients with these tumors have an excellent prognosis, a small percentage experience recurrence after local therapy alone. During the past two decades, numerous prognostic markers have been extensively evaluated in node-negative IBC in general. However, only a few studies have evaluated prognostic factors specifically in T1a,bN0 IBC; therefore, the use of adjuvant therapy in these patients is controversial.
Design: We reviewed the histologic sections of 273 patients with T1a,bN0 IBC treated at MDACC between 1980-1999 to determine microscopic tumor size; multifocality; histologic subtype; nuclear grade; the presence, type, and percentage of associated DCIS; and the presence of FCC with and without atypia, and lymphovascular invasion. Kaplan-Meier survival curves were used to evaluate overall survival (OS) and disease-free survival (DFS).
Results: The median patient age was 58 years (range 29-86). Patients had undergone breast-conserving surgery (66%) or mastectomy (34%). Median follow-up was 8.5 years. At 10 years, the DFS and OS rates were 92% and 86%, respectively. Median tumor size was 8.0 mm (range 1.1-10). Types of IBC included invasive ductal ca.(81%), prognostically favorable ca. (tubular, mucinous and cribriform) (8%), lobular ca. (4%) and mixed types (7%). 21% of patients had extensive DCIS (>50%), and 30% had grade 3 DCIS. The grade and presence of extensive DCIS were significantly associated with DFS: patients with lower grades had longer DFS than did patients with higher grades ($p=0.01$). Non-proliferative FCC, proliferative FCC with and without atypia were identified in 80%, 36% and 38% of patients, respectively. The presence of FCC was associated with longer OS. Multifocality of the IBC was an important factor in predicting disease recurrence. Patients without multifocal disease had longer DFS than did those with multifocal disease ($p=0.03$).
Conclusions: The histopathologic evaluation and characterization of lesions associated with IBC have prognostic significance in T1a,bN0. Invasive cancers arising in a background of FCC or low-grade DCIS may have a different molecular or genetic background than do tumors arising in a background of high-grade DCIS. Therefore, in all cases of IBC, associated lesions and multifocality should be assessed thoroughly.

133 Breast Carcinomas with Chondroid Differentiation

K Gwin, DT Wheeler, V Bossuyt, FA Tavassoli. Yale University School of Medicine, New Haven, CT; AFIP, Washington, DC.

Background: Metaplastic carcinomas (MC) of the breast reflect a heterogeneous group of neoplasms including some with mesenchymal differentiation. In 2003, the WHO emphasized separation of the histological subtypes to better establish the clinical behavior, prognosis and response to therapy for each variant. Due to their rarity, however, there is limited literature available on the predictive and prognostic factors of the individual subtypes. We undertook a comprehensive review of breast carcinomas with chondroid differentiation to further define its clinicopathological features.

Design: 19 breast carcinomas with chondroid differentiation were reviewed. All cases with clear-cut chondrosarcoma component that failed to immunoreact with any epithelial markers were excluded. Immunohistochemical stains for ER, PR, HER2/neu, AR, EGFR, p63, S100 and Calponin were performed. Clinical follow-up was obtained. The axillary LN were reviewed in 9 cases, and distant metastatic material was available for review in 1 case. The histopathological features and immunohistochemical expression profile were evaluated.

Results: The mean age was 50.3 years (range 35-73 yrs); the mean tumor size was 2.6 cm (range 0.8 - 8.0 cm). Positive LN were present in 55.5%. Among those with positive LN, 60% revealed chondroid differentiation. At the time of initial diagnosis, no patient had distant metastasis. All tumors had chondroid differentiation with positivity for S100, p63 and Calponin. The majority of tumors were well-circumscribed with extensive central necrosis present. The tumor were mainly invasive with a DIN/DCIS component evident only in 30%. Focal squamous metaplasia was also noted in multiple cases. Steroid hormone receptor analysis revealed a triple negative immunoprofile for all cases. Additionally, no expression of the AR was identified. Interestingly, EGFR overexpression was present in all examined cases. Follow-up was available in 8 cases (2-156 month). 1 patient had recurrent disease, 3 patients presented with metastatic disease and 3 patients died of disease.

Conclusions: Breast carcinomas with chondroid differentiation are a distinct subgroup of breast tumors. Nodal metastases are common and patients have a high risk of developing recurrences and metastatic disease. The triple negative immunoprofile along with negativity for AR limits treatment options. The high frequency of EGFR expression makes MC with chondroid differentiation a potential candidate for a trial with EGFR inhibitors particularly if mutations are also confirmed.

134 Does Using a Higher Cut Off for the Percentage of Positive Cells Improve the Specificity of HER2 Immunostaining?

O Hameed, AL Adams, DC Chhieng. University of Alabama School of Medicine, Birmingham, AL.

Background: To improve the specificity of HER2 immunohistochemistry (IHC), the College of American Pathologists is developing guidelines for HER2 testing with one proposed interpretation criterion being the requirement for uniform circumferential staining in at least 30% of tumor cells for a result to be considered positive (in contrast to the 10% cut off for most FDA-approved and non-approved tests). Since a review of our in house HER2 IHC results showed a relatively low specificity of protein overexpression (3+) for HER2 amplification, we wanted to see if using such a higher cut off would improve this specificity, especially since, to our knowledge, there are no studies that specifically address this issue.

Design: A retrospective review of all HER2 testing performed at the authors' institution over a one year period was performed to identify breast cancer cases in which both anti-HER2 (Ventana CB11 or Dako A0485) IHC-stained sections were available for review, and results of HER2 amplification by FISH (Ventana) were known. Blinded to the amplification status, 3 pathologists used a multiheaded microscope to concurrently review the IHC-stained sections. Cases were scored as positive for protein overexpression (3+) by using 3 different cut offs, 10%, 30% and 50%, for the proportion of cells with moderate to strong circumferential staining. Results were then compared to the FISH findings to calculate the sensitivity, specificity, concordance, and accuracy rates using these different cut offs.

Results: There were 98 cases that fit the inclusion criteria. The sensitivity, specificity, concordance, and accuracy rates using the Ventana (n = 53) and the Dako (n = 45) antibodies with the different cut offs are shown in the Table.

Antibody/ Cut off	Sensitivity (95% CI)	Specificity (95% CI)	Concordance (95% CI)	Accuracy (95% CI)
Ventana				
10%	69% (39-90)	83% (67-92)	56% (31-79)	79% (66-88)
30%	69% (39-90)	85% (69-94)	60% (33-83)	81% (69-89)
50%	69% (39-90)	85% (69-94)	60% (33-83)	81% (69-89)
Dako				
10%	71% (42-90)	81% (62-92)	63% (36-84)	77% (64-87)
30%	64% (36-86)	87% (69-96)	69% (39-90)	80% (66-89)
50%	57% (30-81)	90% (73-97)	73% (39-93)	80% (66-89)

CI, confidence interval

Conclusions: 1) Using a 30% cut off for the proportion of positive cells slightly increased the specificity of HER2 IHC, the concordance rate with FISH, and its accuracy, 2) using a 50% cut off further increased the specificity and concordance rates of the Dako antibody, but not of the Ventana antibody, and 3) the findings support the proposition of using a 30%, instead of a 10%, cut off for the proportion of cells with uniform circumferential staining for a result to be considered positive.

135 Histopathological and Clinical Characterization of "Triple-Negative" Invasive Mammary Carcinomas

S Hamidpour, M Davis, BF Kimler, S Hull, F Fan, QJ Khan, P Sharma, C Connor, SM Lai, CJ Fabian, P Thomas, WR Jewell, O Tawfik. Kansas University Medical Center, Kansas City, KS.

Background: Molecular profiling studies have provided evidence that breast cancer is a heterogeneous group of diseases that have different prognoses and different responses to therapy. Triple negative (TN) for estrogen receptor (ER), progesterone receptor (PR) and Her-2 tumors are a rare but unique subtype of invasive mammary carcinoma that is classified by gene expression profiling into a "basal like" subgroup. BRCA1 is also noted to be significantly higher in this subgroup of patients. Current endocrine and/or Her-2 targeted therapy are not valid treatment options for those patients. Here we present the clinical features and prognostic markers to further characterize TN tumors.

Design: Clinical and histopathological characteristics of breast cancer patients from 1993 to 2006 were reviewed. Tumor size, grade, histologic type, angiolymphatic invasion, lymph node status, patients' overall survival for a 13 years, biomarker status including ER, PR, p53, epidermal growth factor receptor (EGFR), BCL-2, Her-2, p27, p21 and ploidy status were evaluated. TN status was defined as ER and PR < 10% and Her-2 negative by FISH and immunohistochemistry.

Results: 168/758(22%) tumors were found to be TN. 88% of the TN tumors were invasive ductal. and 75% were grade 3. Compared to non-TN tumors, TN tumors occurred in younger women, were larger in size, were more likely to be lymph node positive, with higher MIB-1 scores. Rate of lymphovascular invasion was similar in TN and non-TN tumors. TN tumors also expressed higher levels of EGFR and higher levels of p53, but expressed lower levels of bcl-2, p27 and p21. Nine of 10 rare known aggressive special histologic types in the entire cohort including apocrine, medullary, metaplastic and squamous were TN. In multivariate analysis MIB-1, EGFR and histologic grade were statistically significant predictors of a tumor being TN. Patients with TN tumors showed poorer overall survival than did patients with non-TN tumors (p=0.0034).

Conclusions: TN tumors represent a unique subset of breast cancer that are high grade, occurring in younger women and have poor overall survival. Clinical and histopathological characterization of these tumors will assist in their identification and help in the development of better targeted treatment strategies.

136 Loss of Expression of Androgen Receptor (AR) May Play a Critical Role in the Transformation from In Situ to Invasive Ductal Carcinoma of the Breast

K Hanley, J Wang, P Bourne, Q Yang, P Tang. University of Rochester Medical Center, Rochester, NY; RTI Health Solutions, Rsearch Triangle Park, NC.

Background: Androgens and androgen receptor (AR) are involved in the pathogenesis of breast carcinoma. However, the functional role and clinical value of AR expression in breast carcinoma have not been clearly defined, especially its relationship with some of the key factors in breast carcinoma such as nuclear grades; cytokeratin (CK)-classification (luminal: CK8 and CK18 +; basal/stem: CL5/6, CK14, and CK17 +; null: all CK -); and ER/HER-2 classification (basal: ER -/HER-2 -; basal-like: ER-/HER-2 +, luminal-A: ER+/HER-2 -; and Luminal-B: ER+/HER-2 +). Different nuclear grades are associated with distinct genetic pathways and biological behavior, while the basal subtypes of both classifications are associated poor clinical outcome.

Design: We performed immunohistochemical studies of CK5/6, 8, 18, 14, and 17 as well as ER, HER2 and AR in 171 cases of breast carcinoma including 77 cases of pure DCIS (34 high grade and 43 non-high grade), and 94 cases of DCIS/IDC (44 high grade and 50 non-high grade) and analyzed the relationship between AR expression and nuclear grades, 3 CK-subtypes (luminal, basal/stem, null), and 4 ER/HER-2 subtypes (basal, basal-like, luminal-A, and Luminal-B).

Results: 1) The rate of AR negativity is significantly higher in high-grade DCIS/IDC (45%) compared to non-high grade DCIS/IDC (8%) and non-high grade (5%)and high-grade DCIS (9%), suggesting that the loss of AR expression may play an important role in the transformation from DCIS to IDC of high-grade carcinoma. 2) In high grade DCIS/IDC, AR negativity is strongly associated with basal/stem subtype (13/15) in CK classification and with basal subtype (16/24) in ER/HER-2 classification, indicating that loss of AR expression is important in the transformation of in situ to invasive CK5/6, 14, 17 positive and ER, HER-2 negative high grade carcinoma. 3) In non-high grade carcinoma (DCIS and DCIS/IDC) The rate of AR positivity is very high (95% for DCIS and 92% for DCIS/IDC) and its expresstion is strongly associated with ER expression.

Conclusions: Loss of AR expression may play an important role in the transformation from in situ to invasive high-grade ductal carcinoma. More studies are needed to further explore the role of AR in breast carcinogenesis and the possiblity of AR as an potential therapeutic target for breast carcinoma.

137 Estrogen Receptor Co-Activator (AIB1) Protein Expression by Automated Quantitative Analysis (AQUA) in a Breast Cancer Tissue Microarray (TMA) and Association with Patient Outcome

M Harigopal, J Heymann, RL Camp, DL Rimm. Yale Univ. School of Medicine, New Haven, CT.

Background: Amplified in breast cancer (AIB1 or SRC-3) is an estrogen receptor (ER) regulatory protein that binds estrogen (E) response elements to mediate transcriptional activity of target genes. AIB1, together with other co-activators like transcription intermediary factor 2 (TIF2) and nuclear receptor co-repressor (NCoR), is implicated in E signaling pathway and E regulated tumor progression. We investigated the prognostic significance of AIB1, TIF2 & NCoR protein expression, and studied their relationship to prognostic biomarkers like ER, progesterone PR, & HER-2, as well as between AIB1, TIF2 & NCoR.

Design: AIBI, TIF2 & NCoR were studied by fluorescent immunohistochemical staining of a TMA (670 breast cancer and 20 normal samples) and analyzed by an objective method (AQUA). Cytokeratin was used to identify tumor cells. Targets (AIB1, TIF2 & NCoR) within the tumor were measured by Cy-5 tyramide.
Results: Using Cox univariate survival analyses of the AQUA scores, high AIB1 expression was associated with poor outcome (p = 0.002), while no association was noted for TIF2 (p=0.376) & NCoR (p = 0.12). AIB1 was studied at two fold redundancy to check reproducibility (Rho=0.4). The average score was used for AIB1 expression in 560 analyzable tumors. X-tile software was used to select training and validation sets (n=280 in each), and to find optimal cut-points on the training set. This cut-point (top 55% vs bottom 45%) in the validation set showed that high AIB1 is associated with poor survival (log rank p = 0.005). When subclassified by nodal or ER status, AIB1 was not prognostic in the node+ subset (p=0.07). The ER+, ER– and node– subsets showed high AIB1 expression to be associated with poor outcome (p=0.014; p=0.019; p=0.007 respectively). AIB1 retained its independent association with survival by multivariate analyses (p=0.028). There was a low but significant correlation between AIB1 & ER (Rho=0.18) and AIB1 & PR (Rho=0.11) but AIB1 & HER2 were not correlated (Rho=0.05). There was significant correlation between AIB1 & NCoR (Rho=0.56, p< 0.001), AIB1 & TIF2 (Rho=0.453, p< 0.001) and NCoR & TIF2 (Rho=0.562, p<0.001).
Conclusions: High expression of ER co-activator AIB1 in breast cancer is associated with poor patient survival. There was significant correlation between AIB1 and ER & PR biomarkers and between AIB, TIF2 & NCoR. AIB1 could be valuable to classify node– patients and also in predicting response to endocrine therapies.

138 Magnetic Resonance Image Guided Breast Core Biopsy Findings in High and Low Risk Patients

M Harshan, D Aslan, M Vazquez. New York Presbyterian Hospital-Weill Cornell, New York, NY.
Background: Magnetic Resonance Imaging (MRI) is a highly sensitive technique for the detection of breast cancer, especially in women with a personal or family history of breast cancer or dense breast parenchyma. Often there is neovascularity on MRI which causes mass or non-mass enhancement of the tumor after the injection of gadolinium, even in the absence of mammographic and ultrasound findings.
Design: A retrospective review of consecutive MRI guided breast core biopsies (BCB) performed over a period of 19 months was done to evaluate the pathologic diagnoses in high and low risk patients in the absence of mammographic and ultrasonographic findings suspicious for malignancy. The BCB were stratified into high and low risk groups. The morphology of the MRI detected lesions were classified as mass enhancements and non-mass enhancements.
Results: There were 57 MRI guided BCB in 56 patients. The age of the patients ranged from 32 to 78 years. Carcinoma was identified in 7 cases (12.3%), six in the high risk group (85.8%) and one (14.3%) in the low risk group.

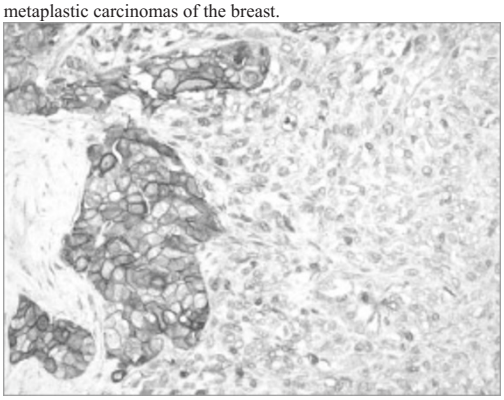
Pathologic Diagnosis	Breast biopsies	Personal history of Breast Ca n=27	Family history of Breast Ca n=12	Low risk group n=18	Mass n=30	Non-mass n=24
Invasive Carcinoma	1 (1.8%)	1 (3.7%)	-	-	-	1 (4.2%)
DCIS	5 (8.8%)	3 (11.1%)	1 (8.3%)	1 (5.6%)	1 (3.3%)	4 (16.7%)
LCIS	1 (1.8%)	1 (3.7%)	-		1 (3.3%)	-
ALH	2 (3.5%)	2 (7.4%)	-		1 (3.3%)	1 (4.2%)
Fibroadenoma	2 (3.5%)	1 (3.7%)	1 (8.3%)		2 (6.6%)	-
Phylloides Tumor	1 (1.8%)	1 (3.7%)	-		1 (3.3%)	-
Papillary Lesions *	6 (10.5%)	3 (11.1%)	-	3 (16.7%)	2 (6.6%)	2 (8.3%)
Other Benign Lesions **	39 (68.4%)	15 (55.6%)	10 (83.3%)	14 (77.8%)	22 (73.3%)	16 (66.7%)
Total	57 (100%)	27 (100%)	12 (100%)	18 (100%)	30 (100%)	24 (100%)

* 2 case unknown enhancement pattern, ** 1 case unknown enhancement pattern

Conclusions: Carcinoma on MRI guided BCB is commonly seen in high risk patients as a non-mass enhancement. The incidence of carcinoma in low risk patients with dense breast parenchyma is low. MRI should be performed to evaluate the extent of carcinoma in high risk patients, even in the absence of mammographic or ultrasound findings. It was not efficacious in low risk patients in this series.

139 Aberrant ß-Catenin Expression Is Associated with Morphology and Prognosis in Metaplastic Mammary Carcinomas

MJ Hayes, A Witkiewicz, CG Kleer. University of Michigan Medical Center, Ann Arbor, MI; Thomas Jefferson University, Philadelphia, PA.
Background: Metaplastic mammary carcinomas are uncommon, with a guarded prognosis and largely unknown molecular etiology. Membrane-bound ß-catenin facilitates intercellular adhesion, while aberrant nuclear expression of ß-catenin may cause transcriptional activation and carcinogenesis. Aberrant ß-catenin expression has been associated with poor prognosis in carcinomas of the colon, pancreas, and larynx. Studies of mostly ductal carcinomas have not found significant aberrant ß-catenin, but metaplastic carcinomas have not been selectively studied.
Design: 31 metaplastic carcinomas were grouped by metaplastic differentiation: squamous, spindle cell, or chondroid. ß-catenin immunostaining used a standard Dako protocol. Normal ß-catenin was defined as crisp membrane staining. Tumors with reduced or absent membrane staining, and/or >5% of tumor cells with nuclear and/or cytoplasmic staining were called aberrant. Clinical outcome at last follow-up visit was recorded.
Results: 91% (28/32) of all metaplastic carcinomas had aberrant ß-catenin. Figure 1 illustrates aberrant expression of ß-catenin in spindle component. 95% (21/22) of tumors with spindled areas had no membrane staining, 68% had nuclear staining, and 36% had cytoplasmic staining. 43% (10/23) of tumors with squamous areas had reduced membrane staining, 26% had nuclear staining, and 48% had cytoplasmic staining. All 3 chondroid cases had cytoplasmic staining, 2 had reduced membrane staining, and none had nuclear staining. Patients with nuclear ß-catenin expression had a mortality rate of 25%, compared to 14% in those without nuclear staining.
Conclusions: Aberrant ß-catenin expression is frequent in metaplastic carcinomas and is highly associated with spindle cell and chondroid areas. The consistent lack of membrane staining in spindled areas suggests a role for activation of ß-catenin in the loss of intercellular cohesion. Increased nuclear expression and increased mortality could be associated with alteration of wnt or other regulatory pathways, suggesting a previously unknown role for ß-catenin induced transcriptional activation in the pathogenesis of metaplastic carcinomas of the breast.



140 Where Were Basal-Like Mammary Duct Carcinoma (BL-MDC) Hiding Prior to the Year 2000?

W He, L Marinescu, RW Cartun, D Stevens, A Ricci. Hartford Hospital, Hartford, CT.
Background: Basal-like mammary duct carcinoma is a recently described form of metaplastic carcinoma originally defined by microarray gene expression analysis. Histologically these tumors are invariable high-grade and are also "triple-negative" (i.e. ER/PR/HER2) by immunohistochemistry (IHC). We postulated that prior 2000, such tumors may have been diagnosed as "atypical medullary carcinoma" or some similar designation. The present study explores this hypothesis.
Design: The Hartford Hospital Surgical Pathology files from 1999-2005 were queried for breast carcinoma cases diagnosed as "atypical medullary carcinoma" (AMC), "medullary-like carcinoma" or "carcinoma with medullary features". Twenty candidate cases were retrieved. Four were excluded because they were positive for ER and/or HER2. The remaining 16 cases (all high-grade) were studied by IHC for basal cell or myoepithelial markers (S100, calponin, p63, CK5/6 & CK17) and also with EGFr antibody. Immunoreactivities were evaluated by a summed score (0-8) of intensity & distribution after Allred (Modern Pathol 1998). EGFr was scored as HER2 IHC assays.
Results: Fourteen of the 16 cases (87.5%) showed basal/myoepithelial phenotype (by CK5/6 IHC) and/or positive EGFr, consistent with BL-MDC. Twelve of 16 (75%) were CK5/6 positive. CK17, p63 and calponin were less sensitive in this application (8/16-50%, 5/16-31% & 3/16-19%) respectively. S100 was more sensitive (16/16-100%) however the ubiquitous presence of Langerhan's histiocytes rendered interpretation difficult.
Conclusions: Before characterization as a special type of metaplastic carcinoma after the year 2000 many BL-MDC were probably diagnosed as "atypical medullary carcinoma" or "carcinoma with medullary features". This recognition could account for prior statements in the literature with regard to AMC and even medullary carcinoma such as a cited poor prognosis for the former and the sometimes-reported S100 positivity in the latter.

Case	S100	Calponin	p63	CK5/6	CK17	EGFr
#1	6.5	0	2.5	3	5.5	3
#2	3	0	0	0	0	0
#3	6.5	0	6	5.5	6.5	2
#4	3	0	0	6.5	5.5	2
#5	7	0	2	6	4	3
#6	3	0	0	5	0	0
#7	2	3	0	6	0	0
#8 *						
#9	6	2.5	0	6	5	1
#10	2.5	0	0	2	0	0
#11 *						
#12	5	0	0	0	0	0
#13	6	0	5.5	6	6.5	3
#14	6	0	0	0	0	3
#15 *						
#16	7.5	0	0	0	0	2
#17	6	0	4.5	5	6.5	2
#18	6.5	5.5	0	6	5	1
#19	6	0	0	3	0	1
#20 *						

(* indicates excluded cases - see Design)

141 A Low Ki-67 Proliferation Index in Atypical Ductal Hyperplasia Diagnosed on Breast Core Biopsy Is Not Associated with the Presence of Ductal Carcinoma In Situ on Subsequent Excision

JW Henderson, SZ Al-Quran, JA Pfeifer, O Hameed. University of Alabama School of Medicine, Birmingham, AL; University of Florida, Gainesville, FL; Washington University School of Medicine, St. Louis, MO.

Background: A diagnosis of atypical ductal hyperplasia (ADH) on breast core biopsy (CBX) is usually followed by an excisional biopsy (EBX) to exclude the presence of a more significant lesion. Recently, it has been shown that Ki67 immunohistochemistry (IHC) can help stratify cases of ADH as to the likelihood of finding ductal carcinoma in situ (DCIS) on subsequent EBX. The aim of this study was to see whether this can be validated by application to a larger number of cases.

Design: A computer database search of the surgical pathology files of 3 major academic medical centers was performed to identify breast CBX material diagnosed as ADH and for which both the slides were available for review, and the pathological findings on subsequent EBX were known. Consecutive histological sections were stained with H&E and by IHC utilizing a Ki67 antibody (clone MM1). One investigator, blinded to the EBX findings, calculated the proliferation index (PI; percentage of Ki67-positive nuclei) in the duct space(s) involved by ADH. Using a previously tested cut off of 2% (AJCP 2005;124:862), cases were then classified as "positive" or "negative." Results of IHC were then compared to the EBX findings to calculate the sensitivity, specificity, negative and positive predictive values (NPV, PPV), and accuracy rates for this method.

Results: Forty-one cases have been identified in which both the diagnosis of ADH was confirmed, and representative material was still present on the recut and IHC-stained sections. Of these, 12 (29%) had a subsequent EBX diagnosis of DCIS. The PI of these cases was significantly higher than those without DCIS on EBX (mean = 13.1% versus 2.1%; $P < 0.001$). At a cut off of 2%, the sensitivity of this method was 100% (95% confidence interval (CI), 70-100%), the specificity was 75% (95% CI, 56-89%), the NPV was 100% (95% CI, 82-100%), the PPV was 63% (95% CI, 39-83%), and the accuracy was 83% (95% CI, 69-91%).

Conclusions: The findings support use of Ki67 immunostaining to help stratify breast CBX cases with a diagnosis of ADH. Specifically, cases with a low Ki67 PI appear unlikely to be associated with DCIS on EBX. Such patients arguably could be spared this additional surgical procedure. More cases are being tested to see if these findings can be further validated.

142 Phenotypic Alterations in Ductal Carcinoma In Situ-Associated Myoepithelial Cells: Biological and Diagnostic Implications

JB Hilson, SJ Schnitt, LC Collins. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Background: Mammary ductal-lobular spaces that are involved by DCIS are surrounded by a layer of myoepithelial cells (MEC). Recent studies using expression profiling and gene methylation assays have indicated that DCIS-associated MEC show a number of important differences from MEC of normal breast tissue. It has been suggested that such alterations in DCIS-associated MEC may be important determinants of the progression of DCIS to invasive breast cancer. The purpose of this study was to further investigate phenotypic alterations in DCIS-associated MEC.

Design: We performed immunostains on paraffin sections of 20 cases of DCIS, including 10 cases each of high grade (HG) and non-HG DCIS, using antibodies to 7 markers of MEC that identify different MEC components including smooth muscle actin (SMA), calponin (calp), smooth muscle myosin heavy chain (SMMHC), p63, CD10, CK5/6 and p75. For each of the 7 MEC markers studied, the staining intensity of the DCIS-associated MEC and the proportion of involved spaces with circumferential MEC staining were compared with internal positive controls consisting of normal ducts and lobules on the same section.

Results: Compared with normal breast ducts and lobules on the same slide, the proportion of cases of DCIS that showed reduced expression of the 7 MEC markers was 79% for SMMHC, 50% for calp, 29% for CD10, 21% for p75, 19% for CK5/6, 16% for p63, and 0% for SMA. The proportion of cases showing a reduced frequency of circumferential MEC staining compared to normal breast ducts and lobules on the same slide was 42% for SMMHC, 17% for CK5/6, 11% for p63, 7% for CD10, and 0% for SMA, calp, and p75. These phenotypic alterations were seen in MEC associated with cases of both HG and non-HG DCIS.

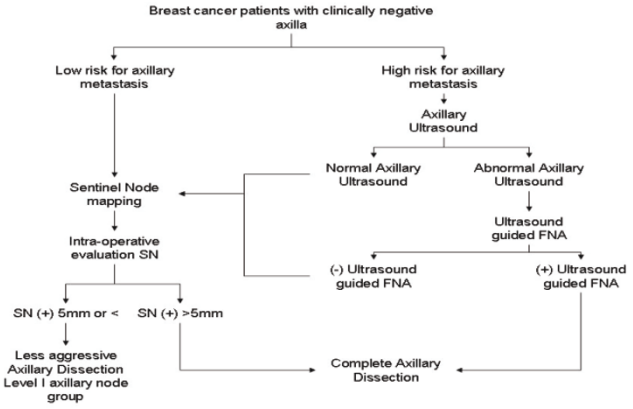
Conclusions: DCIS-associated MEC often show phenotypic differences from normal MEC. In particular, expression of SMMHC is commonly reduced in DCIS-associated MEC relative to normal MEC. Whether or not altered expression of these MEC proteins is biologically important in the progression of DCIS to invasive breast cancer remains to be determined. The biological implications notwithstanding, our results indicate that the expression of these MEC markers in DCIS varies considerably and also differs from their expression in normal MEC. This observation should be taken into consideration when selecting MEC markers for use in clinical practice to distinguish in situ from invasive lesions.

143 Management of the Axilla in Patients with Clinically Negative Nodes: The University of Kentucky Breast Cancer Center Experience

JL Hinson, YM Brill, JT Davis, P McGrath, A Moore, E Romond, LM Samayoa. University of Kentucky, Lexington, KY; VA Hospital, Lexington, KY.

Background: Approximately 1 out of 3 patients with clinically negative (-) axillae will undergo complete axillary dissection (CAD). A high percentage of these patients will have a single positive (+) lymph node (LN) often with minimal disease (N1a). The benefit of CAD in such patients is open to debate. This review focuses on restricting CAD to patients at high risk for > 3(+) LN, and proposes a more conservative approach for those patients with a greater likelihood of having limited LN involvement (1 to 3 LN).

Design: Data from two different series were retrospectively reviewed in terms of final number of (+) LN on CAD. Series "A": 467 patients who underwent SN mapping without pre-operative axillary ultrasound (U/S) and series "B": 168 patients who underwent axillary U/S followed by ultrasound guided Fine Needle Aspiration biopsy (U/S-FNA) prior to SN mapping, selected on the basis of variables in their breast primaries, that are associated with a higher incidence of LN metastasis.



Results: Patients with > 3 (+) LN (N2-3) represented 5 -10% of all patients with clinically (-) axillae (Series A & B respectively). Patients with SN tumor deposits measuring 5mm or less carried a 97% chance of having a single (+) LN (A series). 70% of patients at high risk for axillary metastasis had abnormal preoperative axillary U/S. U/S-FNA detected 100% of N2-3 patients. All of the N1a patients detected by U/S-FNA had tumor deposits greater than or equal to 1cm. All of the N1a patients missed by U/S and or U/S-FNA had SN tumor deposits less than or equal to 5mm.

Conclusions: CAD is indicated in patients having a positive pre-operative U/S-FNA and in patients with SN tumor deposits >5mm at the time of surgery. A conservative axillary dissection may suffice in patients with normal pre-operative U/S of the axilla or (-) U/S-FNA, provided that at the time of surgery their SN is (-), or harbors tumor deposits measuring 5mm or less. Following this practice, the overall risk for complications associated with CAD could be reduced from 30 to 10%.

144 Sentinel Lymph Node Biopsy in Breast Cancer Patients More Than 70 Years of Age

M Hulvat, K Yao, J Norton, G Aranha, PB Rajan. Loyola University Medical Center, Maywood, IL.

Background: The management of the axilla is controversial in elderly patients more than 70 years of age, especially in those who have small breast cancers. Sentinel lymphadenectomy in this population of breast cancer patients may have different histopathologic results than in the non-elderly population.

Design: This is a retrospective review of 348 breast cancer patients who underwent sentinel lymphadenectomy at our institution between 2000-2005. This included 96 breast cancer patients with age more than 70 years. Pearson x2 analysis and the Z test of column proportions were used to compare histopathologic findings of sentinel and axillary lymphadenectomy in breast cancer patients less than 70 years versus more than 70 years of age.

Results: A total of 348 patients were analyzed. Ninety-six (26.8%) patients were more than 70 years of age. Of these 96 patients, 31 (31.6%) had a tumor positive sentinel node (SLN) and 65 (68.4%) were tumor negative. Only six patients (6.4%) had tumor positive non-SLNs. The number of patients with a tumor positive SLN was greater in the patients <70yo vs ≥70yo (42.7% vs. 31.6%) and it trended towards significance, ($p=0.057$). The number of patients with tumor positive non-SLNs was significantly greater in patients <70yo vs ≥70yo (17.4% vs. 6.4%, $p=<0.05$). When patients with a tumor size of 1-2cm were examined, the incidence of tumor positive SLNs and non-SLNs was significantly higher in patients <70yo vs ≥70yo (41.1% vs. 23.1%, 14.9% vs. 2.6% respectively, $p<0.05$).

Conclusions: Sentinel lymphadenectomy in elderly breast cancer patients with small breast cancers shows a lower rate of nodal metastases. Larger studies are needed to determine if sentinel lymphadenectomy can be omitted for breast cancer patients more than 70 years of age who have small cancers.

145 Lobular Neoplasia (Atypical Lobular Hyperplasia and Lobular Carcinoma In Situ) in Core Biopsy Specimens: When Is Excision Necessary?

H Hwang, LD Barke, CL Schiller, EB Mendelson, B Susnik. Northwestern University, Chicago, IL.

Background: Standardized recommendations for the management of lobular carcinoma in situ (LCIS) or atypical lobular hyperplasia (ALH) in core biopsy specimens (CB) are not established. The diagnosis of LCIS or ALH in core biopsy is often followed by a subsequent excision (EX) of the CB site. Previous reports on frequency of ductal carcinoma in situ (DCIS) or invasive carcinoma (IC) in subsequent EX specimens of lobular neoplasia (LN) vary from 3% to over 25%. The aim of the current study was to review pathology and imaging studies of patients with ALH or LCIS diagnosis on CB and define morphological features associated with malignancy (DCIS or IC) in subsequent EX.

Design: With IRB approval we reviewed 331 consecutive CB with a diagnosis of ALH or LCIS from 1996-present. These CB were negative for IC or DCIS. The lobular nature of lesions with a non-classic morphology was confirmed with E-cadherin immunohistochemical stain. Lesions associated with atypical ductal hyperplasia (ADH) were grouped separately. Detailed review of imaging findings was performed in cases that had DCIS or IC in subsequent EX.

Results: EX was performed in 126/331 LN cases. These 126 cases included 45 LN+ADH and 81 pure LN (53 ALH and 28 LCIS). LN+ADH were associated with DCIS and/or IC in EX in 20% (9/45). Pure LN (3 ALH and 5 LCIS) were associated with DCIS or IC in EX in 9.9% (8/81).

LN in core biopsy specimens: findings in subsequent excisions

CB diagnosis	DCIS or IDC in excision
LN+ADH	20.0% (9/45)
pure LN	9.9 % (8/81)*

*Discordant imaging/ pathology was present in 6/8 cases.

Discordance of imaging and pathology was present in 6/8 cases: mass (2), architectural distortion (1), MR enhancement (1), calcifications not adequately sampled (1), pleomorphic calcifications (1). The two remaining cases with concordant imaging and pathology had florid LCIS with necrosis and were associated with microinvasive carcinoma in one and DCIS in the other EX.

Conclusions: When ALH or LCIS is diagnosed in CB, without associated mass lesion and with concordant imaging/pathology, there is a 3% upgrade to DCIS or IC in our excisional specimens. The upgrades in our study is limited to cases with florid LCIS with necrosis. Excision may not be necessary when ALH or classic LCIS with concordant imaging/pathology is identified in CB.

146 Epithelial Inclusions in Axillary Lymph Nodes: A Histological and Immunohistochemical Study of Eight Cases

JD Jakowski, S Suster. The Ohio State University Medical Center, Columbus, OH.

Background: Epithelial inclusions (EI) in axillary lymph nodes (ALN) is a rare occurrence that can introduce serious diagnostic difficulties, particularly when found in patients with an established history of breast cancer. The aim of our study was to evaluate a series of cases of EI in ALN and define their histopathologic and immunohistochemical (IHC) features, compare them to those reported in the literature, and propose criteria to aid in their proper identification.

Design: We reviewed eight cases of women who had EI in one of their ALN. A broad IHC panel of stains was investigated including estrogen (ER) and progesterone (PR) receptors, smooth muscle actin (SMA), p53, Ki-67, cyclooxygenase-2 (COX-2) and BRST-2 antibodies.

Results: The histological criteria favoring benign EI included a lack of architectural and cytological atypia, absence of stromal desmoplasia or lymphovascular invasion, and an appearance dissimilar to that of the synchronous breast carcinoma. Other clues to their benign nature included secondary changes such as apocrine or squamous metaplasia and cystic degeneration. Results of our IHC study showed an almost complete absence of Ki-67 and COX-2 expression in the epithelial cells of benign EI, in contrast with high expression in the tumor cells in control cases of metastatic carcinoma to ALN. One of our cases, however, showed strong focal overexpression of COX-2 in a benign EI with fibrocystic-like changes that could pose a potential pitfall for diagnosis. SMA was also of value in separating benign EI from metastatic carcinoma, by showing a distinctive pattern of staining of the stroma surrounding the cell nests. ER and PR status, BRST-2, and p53 staining were not useful in most cases to discriminate between a benign EI and a grade 1 or 2 metastatic breast carcinoma.

Conclusions: To our knowledge this is the largest series describing the histopathology and IHC of EI in ALN. The finding of a myoepithelial layer has been traditionally regarded as strong supportive evidence of a benign nature in EI; however, none of the cases of benign EI in our study demonstrated a myoepithelial cell layer either by histology or on IHC staining. Therefore, the absence of a myoepithelial cell layer in an EI in an ALN should not be regarded as synonymous with malignancy. Identification of benign EI in ALN remains a diagnostic problem for pathologists with serious clinical implications. Use of a combined approach that includes morphologic and IHC features may be of help in making this distinction.

147 Measurement of Her-2/neu Membrane Receptor Immunoreactivity by Fully Automated Digital Image Analysis

RE Jimenez, AS Basu, S Mahooti, AS Gholap, SH Barsky. The Ohio State University College of Medicine, Columbus, OH; BioImagene, Inc, Cupertino, CA.

Background: Her-2 / neu staining produces colorimeteric differences in membrane staining which are interpreted by pathologists and expressed on an ordinal scale of relative staining from 0 – 3+. The Her-2 / neu status represents important prognostic / predictive information of human breast cancer but its subjective interpretation results in interobserver, intraobserver and fatigue variability. Semi-automated image analysis systems have been used with limited success to eliminate this variability but still require the selection of the regions of interest by a pathologist. In addition, because of a significantly high percentage of false negatives and false positives by the subjective immunocytochemical method of Her-2 / neu assessment, Her-2 / neu is also measured by fluorescence in situ hybridization (FISH), which is considered the gold standard.

Design: To address all of these limitations, we created fully automated and pathologist-independent algorithms to analyze pixels of virtual slides. These algorithms could successfully divide a virtual slide into a grid of hundreds of fields of view (FOV), analyze each field and identify breast cancer cells in a background of stroma, successfully calculate the epithelial percentage in each field, determine the FOV with the highest density of cancer cells and select those FOV for further study. Image acquisition utilized scanners capable of producing images with a resolution of 20 pixels /10µ. Selected FOV were processed by jpg conversion of scanned virtual slides, screened for quality, enhanced and processed.

Results: Membrane algorithms utilizing both colorimetric (RGB) as well as intensity (gray scale) determinations were used to quantitate Her-2 / neu immunoreactivity. The algorithm-based ordinal values for Her-2 / neu strongly correlated with the subjective measurements (Intraclass Correlation: 0.88; 95% Confidence Interval: 0.79, 0.94) yet exhibited no interobserver, intraobserver or fatigue variability. In addition the algorithms provided measurements of Her-2 / neu on an expanded continuous scale that subjective review could not assess. These algorithm continuous scale Her-2 / neu measurements more strongly correlated with Her-2 / neu FISH than the subjective measurements.

Conclusions: Because of all these properties, fully automated digital image analysis of Her-2 / neu immunoreactivity "adds value" as a means of measuring this important membrane biomarker of human breast cancer.

148 Quantitative Image Analysis (QIA) of HER2 Immunohistochemistry (IHC) Provides a Reproducible, Accurate, and Cost-Effective Tool for HER2 Assessment of Breast Cancers

RL Judd. Ameripath Florida, Orlando, FL.

Background: Both IHC and FISH are utilized for HER2 testing of breast cancer. Reviewers of HER2 clinical trial data have noted problems with manual IHC interpretation. This has resulted in recommendations to perform primary FISH testing on all cases. However, HER2 IHC with QIA has not received appropriate consideration.

Design: This report details 63 consecutive breast cancers that had HER2 IHC quantified by the Ventana Image Analysis System (VIAS) and reflex HER2 FISH due to an equivocal IHC score or a specific request from the clinician. Each of the 63 slides was quantified in quadruplicate in a blinded manner, with the standard deviation (SD) calculated for each set of 4 scores and the root mean square average SD calculated for the complete set of 63. Average VIAS scores were plotted versus FISH ratios and the resulting scatter plot analyzed to determine the optimal equivocal score range in this test population. Finally, a cost-benefit analysis was performed, comparing VIAS-reflex FISH with universal FISH.

Results: The SD for the 63 quadruplicate sets ranged from .028 to 0.323, with a root mean square average of 0.134. In this cohort of 63 cases, all 6 VIAS scores >2.7 were FISH positive, and all 17 VIAS scores <1.4 were FISH negative. In our total patient population, 10% of breast cancers scored >2.7, 78% scored <1.4, and 12% fell between 1.4 and 2.7. From a cost-benefit perspective, estimating charges for 88365X2 at 3 times those for a single 88361, and using 12% as the FISH reflex rate, the VIAS-FISH protocol would provide the same accuracy as universal FISH, but at only 45% of the cost.

Conclusions: Although the number of cases is certainly limited in this preliminary report, these results suggest that screening of breast cancers with HER2 IHC and QIA, followed by reflex FISH for equivocal results, can provide the best combination of reproducibility, accuracy, practicality, and cost-effectiveness. The average SD of 0.134 is comparable to what has been reported for FISH cases near the 2.0 cut-point ratio, confirming excellent reproducibility. The accuracy of QIA allows us to focus reflex FISH testing and target only those cases that will truly benefit from the added expense. This reduces the test burden on the FISH laboratory without sacrificing any of the accuracy of universal FISH. It also results in a savings of approximately 55% versus universal FISH testing.

149 LCIS Is Often Composed of Multiple Distinct Subclones Despite Its Monomorphic Appearance: Evidence for Clonal Divergence

R Kanhere, NS Goldstein. William Beaumont Hospital, Royal Oak, MI.

Background: LCIS is traditionally viewed as single monoclonal neoplastic process. Grade 1 DCIS is a genetically diverse process composed of multiple subclones of neoplastic cells. LCIS and pure-grade 1 DCIS share many of the same background molecular alterations. Little is known about the extent of clonal diversity within LCIS. The goal of this study was to evaluate the extent of genetic diversity and clonal heterogeneity within LCIS.

Design: 20 cases with abundant LCIS in association with <2 cm invasive classic lobular or grade 1 ductal carcinoma were retrieved. LCIS was microdissected out of 8 blocks containing pure LCIS in each case. DNA was extracted and amplified using PCR to 21 microsatellite markers, 6 of which were centered around the *CDH1* E-cadherin gene (16q21.1). Four markers were centromeric to or spanned the E-cadherin gene, one overlapped and extended beyond (D16s520) and one was completely telomeric to the gene (D16s422). Amplicons were analyzed with capillary electrophoresis. LOH for each marker was defined as +/-50% of the of normal tissue allelic ratio. A LOH mutational pattern was established for each block and compared.

Results: LCIS was monoclonal in 9 of 20 (45%) cases and composed of multiple distinct clones in the other 11 (65%) cases. Seven of these nine cases (35%) had two distinct LCIS clones in separate, three cases (15%) had three distinct LCIS clones in separate blocks and 1 case had four separate LCIS clones. The LCIS clones within the same breast in six of the 11 cases shared 1 to 3 non-*CDH1* marker deletions despite the disparate allelic *CDH1* marker LOHs. 12 cases (60%) had at least 1 block in which no LCIS clone was identified. Three LCIS clones from 3 separate polyclonal LCIS cases had allelic deletions in D16s422. The LCIS cells of these lesions were larger, slightly more pleomorphic, and had more cytoplasm that LCIS cells of lesions with allelic deletions of markers within *CDH1*.

Conclusions: Despite its monomorphic appearance, LCIS is often composed of subclones of neoplastic cells. These results are similar to those of grade 1 DCIS draws LCIS and grade 1 DCIS closer together. LCIS subclones often had a shared set of mutations, suggesting they represent clonal divergence during progressive genetic evolution. The 3 LCIS lesions with allelic deletions in the distal *CDH1* were cytologically similar to the type-B LCIS cells of Haagensen and Lattes and were different from cytologically classic type-A LCIS cell lesions with deletions within or across the entire *CDH1*.

150 *Topoisomerase II-Alpha* and *HER2* Gene Co-Amplification as a Predictor of Response to Therapy and Survival in *HER2* Positive Metastatic Breast Carcinoma

A Kapoor, A Brufsky, S Chow, M Rosenzweig, U Surti, R Bhargava. Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: Preliminary data from a phase III trial of adjuvant traztuzumab (BCIRG, SABCS 2005, abstract 1045) suggests that co-amplification of *HER2* and topisomerase II alpha (*TOP2A*) genes on chromosome 17 results in improved disease free survival benefit from chemotherapy regimens containing both and anthracycline and traztuzumab. We sought to determine if co-amplification of *HER2* and *TOP2A* was a predictor of response and benefit (or lack thereof) to Herceptin® containing chemotherapy in the metastatic setting.

Design: Our institution maintains an ongoing database/tissue bank of over 600 women treated for metastatic breast cancer (MBC) at our center from 1999 to the present. Approximately 100 of these women have breast cancer positive for HER2. We sought to evaluate *HER2* and *TOP2A* gene amplification by fluorescence in situ hybridization (FISH) in primary breast cancers from these women, and to determine if *HER2* and *TOP2A* co-amplification were associated with decreased time to progression (TTP) with first line chemotherapy for metatstatic disease, as well as decreased survival from metastatic disease (overall survival; OS). We have performed a pilot analysis on 21 *HER2* positive cases. Of these cases, 13 were treated with first line docetaxel/carboplatinum/traztuzumab (TCH), 11 of which were on a phase II trial of TCH as first line therapy for MBC (Brufsky, et al., Proc ASCO 2003; 22: 71a.). A tissue mircoarray (TMA) with 3-fold redundancy was constructed using 0.6mm cores from all cases. Four microns thick TMA sections were used for *HER2* and *TOP2A* FISH analysis (HER2/CEP 17 and TOP2A/CEP 17 dual color probes from Vysis Inc. Downers Grove, IL). *HER2* (or *TOP2A*) gene to chromosome 17 ratios of 2.0 or more was considered as amplification.

Results: Of the 21 patients, 6 were co-amplified for TOPO2A and HER2. The median time to progression (TTP) for first line MBC chemotherapy and traztuzumab for the *HER2/TOPO2A* co-amplified versus non co-amplified cases was 11 months versus 17 months (p=0.27) respectively; and the OS from diagnosis of metastatic disease to death was 43 months versus 29 months (p=0.75) respectively.

Conclusions: In this pilot analysis, the TTP and OS difference between the *TOP2A* and *HER2* gene co-amplified group versus the non co-amplified group are not statistically significant. The study is currently ongoing for a larger number of cases.

151 WT-1 Expression in Primary Invasive Breast Carcinoma: Occasional Expression in Micropapillary and Mucinous Subtypes

R Karabakhtsian, R Bhargava. University of Kentucky Medical Center, Lexington, KY; Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: In a workup for carcinoma of unknown primary, strong WT1 expression virtually excludes a breast primary. However, different subtypes of invasive breast carcinomas (IBC) have not been comprehensively analyzed for WT1 expression. Occasional reports of WT1 staining in micropapillary subtype of IBC triggered this study.

Design: Twenty-six primary IBC either reported as "micropapillary" (MP) or with "MP features" were retrieved from the pathology case files. All excision specimens were reviewed and classified as IBC pure MP type (i.e. tumors that showed MP architecture and "reverse polarity" by EMA; n=12), mixed MP and mucinous type (i.e. distinct MP and mucinous morphology and "reverse polarity" seen with EMA; n=2), mixed no special type and MP (i.e. tumors with MP features and only a portion of tumor showing "reverse polarity" with EMA; n=6) and no special type carcinoma with retraction artifact (morphology not highly suggestive of MP architecture and lack of "reverse polarity" by EMA; n=6). A tissue microarray (TMA) with 3-fold redundancy was created for the above cases, with each core diameter of 0.6 mm. The TMA was subjected to immunohistochemical (IHC) stains for EMA and WT1. In addition, a 4 microns section of another TMA of 63 consecutive IBC (54 ductal and 9 lobular) cases was stained with WT1. These 54 ductal carcinomas contained 33 no special type (NST), 1 pure mucinous, 1 pure MP, 2 mixed MP and NST, and 17 other special type of carcinomas.

Results: Reverse polarity with EMA was seen in all 20 cases showing MP architecture. WT1 staining (moderate intensity nuclear staining in 70% of the tumor cells) was seen in 2 of the 20 (10%) carcinomas with MP architecture. Both cases were mixed MP and mucinous carcinomas and staining was seen in both components. All 6 cases of NST carcinoma with retraction artifacts were negative for both EMA (lack of reverse polarity) and WT1. TMA containing 63 cases of consecutive IBC showed WT1 staining in only one carcinoma, which demonstrated mucinous morphology.

Conclusions: Moderate intensity WT1 staining can be rarely seen in primary breast carcinomas that show MP and mucinous morphology. WT1 staining in a tumor at extra-mammary site with MP pattern does not always exclude a breast primary. The frequent association of MP and mucinous carcinomas, their similar IHC profile ("reverse polarity" seen with EMA and occasional WT1 positivity), suggest a close biologic relationship between these two subtypes.

152 Clinical, Histopathologic, and Biologic/Molecular Features of Microglandular Adenosis with Transition into In-Situ and Invasive Carcinoma

I Khalifeh, C Albarracin, Y Wu, N Sneige. M. D. Anderson Cancer Center, Houston, TX.

Background: Microglandular adenosis (MGA) of the breast is a benign lesion that can mimic invasive carcinoma. Histologically, it is characterized by an infiltrative growth pattern and glands lacking a myoepithelial layer. Although carcinoma arising from MGA have been described, it is not clear which cases of MGA will progress to carcinoma, and criteria to distinguish MGA with atypia (AMGA) from MGA involved by carcinoma (MGACA) are lacking.

Design: To better define criteria for differentiating between MGA, AMGA, and MGACA, we evaluated morphologic and prognostic markers in cases of MGA with transition to carcinoma. We obtained 64 cases designated MGAin a search of the Pathology database (1983 to 2006). Only 10 of the 64 cases fulfilled morphologic and immunohistochemical (S-100+, SMA-) diagnostic criteria of MGA, AMGA, or MGACA. Cases were evaluated by immunohistochemistry for cell cycle markers (p53 and Ki-67) and markers for basal-like differentiation (ER, HER2, EGFR, C-kit, CK5/6, CK18, laminin, type IV collagen). Radiological features and clinical follow-up were evaluated.

Results: The 10 cases included six MGA and four AMGA with transition to MGACA. Mean patient ages were 60 y for MGA and 50 y for MGACA. Five of the six MGA cases were microscopic and were incidental and one case presented de novo as a palpable mass. None recurred at follow-up (1–19 y). Follow-up was available for three of four MGACA cases, one with disseminated disease died 2 years of diagnosis, and two had no recurrence within 2 years of diagnosis. All four cases with an invasive component showed transition from MGA to AMGA to invasive carcinoma. The carcinomatous component was metaplastic (one case), adenoid cystic (one case) and ductal carcinomas, NOS (two cases). All cases expressed S-100 and CK8/18, and did not express CK5/6, ER, PR, or HER2 and c-kit. EGFR was diffusely expressed in all six MGA cases and focally expressed in the four MGACA cases. Laminin was more intense in MGA cases and was attenuated in AMGA and MGACA. Ki-67 index was <1% in MGA, readily identified in AMGA, and nearly 100% in the MGACA.

Conclusions: MGA is an extremely rare lesion with a significant rate (40%) of associated atypia or carcinoma. MGA cases had a lower proliferative index (<5%) than AMGA and MGACA. All MGACA cases in our study were triple negative, consistent with a predominantly basal phenotype. Because all cases of invasive carcinoma developed from AMGA, complete excision of AMGA is warranted.

153 Outcome of Neoadjuvant Chemotherapy in Basal-Like Breast Carcinoma: Tissue Microarray Study

JY Kim, DH Shin, KU Choi, HW Kim, HS Kwak, YT Bae, MY Sol. School of Medicine, Pusan National University, Busan, Republic of Korea.

Background: DNA microarray profiling studies have categorized invasive breast carcinomas into hormone receptor positive (HR), HER-2 positive, basal-like and null types. Among them, basal-like carcinoma type has veen known to be associated with the worst clinical outcome. However, the outcome associated with neoadjuvant chemotherapy of basal-like carcinomas compared with other subtypes have not been described in Korean population.

Design: A tissue microarray was constructed from 157 resected breast cancer specimens acquired after neoadjuvant chemotherapy (cyclophosphamide-methotrexate-5FU or cyclophsphamide-adriamycin) from 2002 to 2003 and stained for ER, PgR. Her2, CK8/18, CK5/6, CK14, smooth muscle actin, p63 and CD10. Tumor response was deinfed as PR (partial remission), stable and PG (progression) by criteria of WHO.

Results: All patients were women who ranged in age from 26-74 years, with a mean age of 48 years. The mean size of the overall tumor was 2.5cm (range 0.5cm-7cm). Among 157 cases, 47 cases were hormone-receptor positive, 34 cases were HER-2 positive, 51 cases were basal-myoepithelial marker positive, and 25 cases were null type. In lymph node positive group of the basal-like subtype, the rates of PR and stable were relatively low and that of PG was high than other types. But, in lymph node negative groups of basal-like subtype, the response rates were not significant.

Conclusions: The basal type of invasive ductal carcinoma showed poor response to neoadjuvant chemotherapy in lymph node positive group than HER-2 overexpression type and null type. But, in lymph node negative group, the response rates were not significantly different among HR positive, HER-2 positive and basal-like subtype. Therefore, in triple (ER, PR and HER-2) negative breast cancer, identifying the basal-like subtype can be used for predictive factors of neoadjuvant chemotherapy.

		LN Positive			LN Negative	
	PR	Stable	PG	PR	Stable	PG
HR positive	20.0% (4/20)	50.0% (10/20)	30.0% (6/20)	14.8% (4/27)	66.7% (18/27)	18.5% (5/27)
HER-2 positive	14.3% (2/14)	78.6% (11/14)	7.1% (1/14)	15.0% (3/20)	70.0% (14/20)	15.0% (3/20)
Basal-like	8.3% (1/12)	58.3% (7/12)	33.3% (4/12)	12.8% (5/39)	69.2% (27/39)	17.9% (7/39)
Null	7.1% (1/14)	78.6% (11/14)	14.3% (2/14)	36.4% (4/11)	63.6% (7/11)	0.0% (0/11)

154 Extremely High Concordance between Immunohistochemistry and FISH Testing for HER2 Status Using Subtraction Methodology Scoring in Breast Cancer: A Study of 5942 Cases

PM Kim, LC Goldstein, TS Barry, SJ Kussick, PL Kandalaft, AM Gown. PhenoPath Laboratories and IMPRIS, Seattle, WA.

Background: Accurate HER2 testing is critical in the analysis of breast cancer. Some studies have shown significant discordance of HER2 status between immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) results. A previous study, using alcoholic formalin-fixed tissue from a single institution, showed a dramatic increase in concordance of HER2 status between IHC and FISH when a normalized or subtraction scoring methodology was used (Jacobs TW et al, J Clin Oncol 17:1983-87, 1999). This study expands on these previous findings to include a large sample size from many hospitals with standard tissue fixation to confirm that a high concordance rate between IHC and FISH can be achieved with this scoring methodology.

Design: From January 2003 to August 2006, HER2 status for 5942 breast tumor specimens from more than 100 hospitals in 29 states were determined by IHC and FISH. For IHC, two scores for each specimen were obtained: the non-normalized score (0 to 3+) of invasive tumor, and the normalized score using a subtraction methodology in which the level of staining of non-neoplastic epithelium (0 to 3+) was subtracted from

the level of staining seen in invasive tumor cells. HER2:CEP-17 ratios were obtained by FISH quantitative image analysis (MetaSystemsTM) and compared with the normalized and non-normalized IHC scores to determine respective concordance rates.
Results: Among the 5942 tumors tested by FISH, 1728/1747(98.9%) showing normalized IHC results of 0, 1+ were FISH non-amplified, and 477/505 (94.5%) of IHC 3+ were FISH amplified. Using a non-normalized IHC scoring system, 250/794 (31.5%) of the IHC 3+ cases proved to be FISH non-amplified, whereas using the normalized scoring system only 28/505 (5.5%) of IHC 3+ cases proved to be FISH non-amplified. Importantly, for cases with non-normalized IHC of 0 or 1+ (negative), 15/1514 (1.0%) were amplified and 19/1747 (1.1%) of normalized IHC were amplified.
Conclusions: Using a normalizing IHC scoring system dramatically reduced the incidence of false positive IHC 3+ that proved to be non-amplified by FISH while maintaining nearly identical sensitivity. An extremely high concordance rate between IHC and FISH for HER2 status can be achieved with a subtraction methodology scoring system independent of fixation and tissue processing.

155 Stromal Expression of CD34 and 14.3.3σ May Differentiate Phyllodes Tumor from Fibroadenoma

DF Klink, MO Idowu, MM Grimes, JL Ware. VCU Medical Center, Richmond, VA.
Background: Phyllodes tumor (PT), an uncommon biphasic tumor of the breast, may sometimes be difficult to differentiate from a fibroadenoma (FA). Furthermore, the biologic behavior of PT is often unpredictable. The goal of this study was to identify markers that may be helpful in differentiating PT from FA.
Design: A tissue microarray (TMA) was constructed which included 15 cases of PT (11 low grade, 4 high grade) and 18 cases of FA. Using a TMA precision instrument made by Beecher Instruments three 0.1cm cores from each case were mounted in a paraffin block. Immunohistochemical staining for CD34, c-kit, bcl-2, p53, p63, and 14.3.3σ (protein involved in cell cycle regulation) was performed. Two pathologists scored the staining using a 0-3+ scoring system. No stain=0; light staining less than the clinical control=1+, moderate staining equal to or greater than the control=2+; strong and diffuse staining =3+. A score of 2+ or greater was considered positive.
Results: Differential expression of CD 34 and 14.3.3σ is identified in the stroma of PT compared to FA (see table 1). CD34 is expressed in the stroma in 11/15 (73%) of PT cases compared to 5/17 (29%) of FA cases. 14.3.3σ is expressed in the stroma in 10/14 (71%) of PT cases and 3/16 (19%) of FA cases. Due to technical issues such as paraffin core dropout during sectioning or absence of epithelium not every core was able to be scored for each stain. No difference is identified in the staining patterns of Bcl-2, p53, p63, and c-kit. (see tables 1 and 2).

CD34, 14.3.3σ, and Bcl-2 expression in PT and FA

	CD34 Epithelium	CD34 Stroma	14.3.3σ Epithelium	14.3.3σ Stroma	Bcl-2 Epithelium	Bcl-2 Stroma
PT	0/11 (0%)	11/15 (73%)	10/14 (71%)	10/14 (71%)	5/12 (42%)	1/15 (7%)
FA	2/17 (12%)	5/17 (29%)	12/17 (71%)	3/16 (19%)	8/17 (47%)	0/17 (0%)

C-kit, p53, and p63 expression in PT and FA

	c-kit epithelium	c-kit stroma	p53 epithelium	p53 stroma	p63 epithelium	p63 stroma
PT	4/12 (33%)	0/12 (0%)	0/12 (0%)	1/15 (7%)	0/13 (0%)	0/15 (0%)
FA	8/17 (47%)	0/17 (0%)	0/16 (0%)	0/17 (0%)	0/15 (0%)	0/15 (0%)

Conclusions: Immunohistochemical staining for 14.3.3σ and CD34 may be useful in differentiating phyllodes tumor from fibroadenoma.

156 Tubular Carcinoma and Grade 1 (Well Differentiated) Invasive Ductal Carcinoma: Comparison of Associated Flat Epithelial Atypia and Other Intra-Epithelial Lesions

LP Kunju, CG Kleer. University of Michigan, Ann Arbor, MI.
Background: Tubular carcinomas of breast are uncommon tumors with an excellent prognosis. Recent studies have described a strong association between flat epithelial atypia (FEA) and tubular carcinomas. The aim of this study was to systematically assess morphologic features and associated intra-epithelial lesions between tubular carcinomas and Grade 1 (well-differentiated) invasive ductal carcinomas (IDC).
Design: All tubular carcinomas diagnosed at our institution between 1998 and 2006 on excision biopsies were retrieved. Grade 1 IDC, matched for size, were also retrieved from our surgical pathology files. We recorded the following parameters: specific morphologic features of tubular carcinomas, including open tubule formation, apical snouts, cellular stroma and low-grade nuclei and also the presence of associated intra-epithelial lesions including FEA, atypical lobular hyperplasia (ALH), lobular carcinoma in-situ (LCIS), atypical ductal hyperplasia (ADH) and ductal carcinoma in-situ (DCIS). Hormone receptor, her-2-neu status and lymph node status were also recorded.
Results: Fourteen pure tubular carcinomas with >90 % tubular structures were compared with 18 Grade 1 invasive ductal carcinomas, matched for size. All tubular carcinomas had characteristic morphology of predominantly angulated glands with open lumina and apical snouts distributed in a cellular myxoid to fibrotic stroma. Of the 14 tubular carcinomas, 8 (57%) had associated FEA, 7 (50%) had ADH with micropapillary features, 3 (21%) had low nuclear grade DCIS and 4(29%) had ALH or LCIS. Notably, none of the 18 Grade 1 IDC was associated with FEA. Three of the 18 (16%) Grade 1 IDC had ADH, 7 (39%) had associated DCIS and 3 (16%) had ALH or LCIS. All tubular carcinomas were positive for ER and negative for her-2-neu over-expression. None of the tubular carcinomas had axillary lymph node metastasis vs. 11% of Grade 1 IDC with axillary lymph node metastasis.
Conclusions: There is a strong association between FEA, micropapillary ADH and tubular carcinomas, which may reflect a biologic progression. In our group, none of the Grade 1 IDC was associated with FEA, which suggests that this feature may be useful in the differential diagnosis of difficult cases, supporting the diagnosis of tubular carcinoma. Despite matching for size, Grade 1 IDC more frequently show her-2-neu over-expression and have a higher incidence of lymph node metastasis than tubular carcinomas. These findings further validate the fact that tubular carcinoma is a distinct tumor entity.

157 Clinicopathological Features of Neuroendocrine Carcinomas (NECs) of the Breast

S La Rosa, S Casnedi, C Riva, F Sessa, C Capella. Ospedale di Circolo and University of Insubria, Varese, Italy.
Background: By 2003 WHO classification, primary NECs of the breast are a group of tumors showing morphological features similar to those of NE neoplasms of the gut and lung and expressing neuroendocrine markers in more than 50% of cells. They are rare lesions, representing about 2-3% of all breast cancers.
Design: The aim of this study was to evaluate the main clinicopathological features of a series of 61 NECs of the breast, and to verify if there are differences in clinical and biological behavior with the more frequent ductal and lobular carcinomas (DCs and LCs). Formalin fixed and paraffin embedded tissue sections were stained with H&E, Grimelius' silver stain and antibodies directed against chromogranin A and B (CgA and CgB), synaptophysin, neuron specific enolase (NSE), CD56, αhCG, serotonin, VMAT1, VMAT2, estrogen receptor (ER), progesterone receptor (PgR), p53, Ki67, c-erb-B2. Clinicopathological data were compared with those of 50 DCs and 33 LCs.
Results: All patients were female with an average age of 72 years (range: 46-82), which was statistically different ($p<0.05$) from that of patients with DC (66 years) and LC (63 years). Tumors consisting of well differentiated cells forming nests or trabeculae separated by a fibrovascular stroma, densely sclerotic in some cases, resembled gut NE tumors. Mitotic count was lower ($p<0.001$) in NECs (mean: 3) than in DCs (mean: 7). All NECs expressed at least one neuroendocrine marker in more than 50% of cells. Grimelius positivity was observed in 97% of the tumors. Immunoreactivity (IR) for CgA was found in 39% cases, for CgB in 75%, for synaptophysin in 92%, for CD56 40% and for NSE in 100% NECs. Serotonin-IR was observed in 8% of tumors, αhCG-IR in 21%, VMAT2-IR in 6%, while no NECs expressed VMAT1. The mean percentage of ER expression was 76%, not statistically different from that of DCs (76%) and LCs (70%). The mean percentage of PgR expression was statistically higher ($p<0.05$) in NEC (59%) than in DCs (39%) and LC (38%), while the expression of p53 and c-erb-B2 were statistically lower ($p<0.01$) in NEC than in DCs and LCs. No differences were observed in relation to Ki67 index between NEC and DCs and LCs. The survival rate of patients with NECs was about 90% at five years of follow-up.
Conclusions: NECs of the breast are a group of tumors showing typical histology, and several clinicopathological differences from DCs and LCs.

158 Mammary Lobules, Ducts and Usual Hyperplasia: An Immunohistochemical Analysis for Basal and Luminal Cell Types

HJ Lee, K Donev, U Raju. Henry Ford Hospital, Detroit, MI.
Background: Recent progenitor cell hypothesis of the breast (Werner Boecker *et al., Lab Invest*. 2002; **82**: 737-745) brought a renewed interest in breast basal/myoepithelial(B/ME) cells and luminal cells(LC), which appear to have different pattern of high molecular weight cytokeratin(HMWK) and low molecular weight cytokeratin(LMWK) expression. According to the scheme, cells of breast epithelium have profiles as follows: progenitor cells(CK5+), glandular cells(CK8/18/19+), ME cells(SMA+), intermediary glandular cells(CK5+ and CK8/18/19+), and intermediary ME cells (CK5+ and SMA+). In this study, we investigated the expression patterns of CK5/6, CK8 and SMA for normal ducts(ND), normal lobules(NL) and usual hyperplasia(UH).
Design: Of 60 cases studied, 32 were normal breasts from reduction mammoplasty and 28 were UH. We prepared micro-array paraffin tissue blocks containing 2-4 cores of each case, and counted the number of total ND(60), NL(76), and UH(64). Immunostains for CK5/6 (HMWK), 35βh11(reactive to CK8, LMWK), SMA, p63, vimentin, and LCA were performed. The staining patterns for B/ME and LC layer of ND, NL and UH were analyzed.
Results: A majority(94%) of the intraluminal streaming proliferative cells of UH demonstrated heterogeneous but strong positivity (60-100%) for CK5/6 while peripheral luminal columnar cells were usually negative or interspersed with a few CK 5/6 positive cells. UH showed strong positivity for CK8 (80-100%) and was negative for SMA. B/ME cells of normal ducts were strongly (80-100%) positive for both SMA and CK5/6 in 92% of ducts. In contrast, all B/ME of normal lobule were strongly positive for SMA (100%) but CK 5/6 positivity was seen only in 24%.
Conclusions: 1. In paraffin embedded tissue with commercially available antibodies, the phenotype of B/ME cells appears to be different in ducts and lobules. a) Most lobular B/ME cells express only SMA and therefore have ME cell phenotype. b) Most ductal B/ME cells express both SMA and CK5/6 and seem to correspond to intermediary ME cell phenotype. 2. The intraluminal streaming proliferations of UH have both CK5/6 and CK8 in somewhat heterogeneous pattern and may correspond to intermediary glandular cells or a combination of progenitor cells and glandular cells. However, in this study, it is unclear whether these are different cells or same cells co-expressing the two types of cytokeratin. 3. Usual hyperplasia does not have ME cell phenotype.

159 Race, Body Mass Index, and the Immunophenotype of Triple Negative Breast Cancer

JC Lee, JL Westrup, LA Stead, CL Rosenberg, TC King. Boston University School of Medicine, Boston, MA.
Background: We have previously reported a significant association between the triple negative (ER-, PR-, HER2-) breast cancer phenotype (Tneg) with both race and body mass index (BMI) in a large (310), ethnically diverse population of breast cancer patients. The previous study shows that Tneg tumors were significantly more prevalent in African-American patients and in patients with lower BMI. We sought to determine whether two phenotypic markers [cytokeratin (CK) 5/6 and EGFR] that have been previously reported in Tneg tumors were expressed in our patient population and whether their expression was related to ethnicity and/or BMI.
Design: All available tissue blocks from Tneg tumors in our database (42) were separately stained with antibodies to CK5/6 (Biocare Medical) and EGFR (DAKO) using a streptavidin-Biotin-HRP detection kit (Biogenex). Stains were scored by two

independent observers for intensity (0-3) and percentage of tumor cells staining. 31 tumors from the study population corresponding to other phenotypes were also stained as specificity controls. Herceptest scoring (0-3) was used for EGFR and Allred scoring was used for CK5/6.

Results: There was substantial interobserver agreement for staining intensity, 83% and 87% for CK5/6 and EGFR respectively (weighted kappa = 0.73 and 0.75). Significant expression of EGFR and/or CK5/6 was observed predominantly in Tneg tumors as expected. No ER+ tumors (23) stained positive for EGFR although 13% were positive for CK5/6. 48% of Tneg tumors were positive for EGFR (2+ or 3+ Herceptest score) and 69% were positive for CK5/6 (Allred score > 4). 43% of Tneg tumors were double positive and 74% were positive for at least one marker. Staining intensities for EGFR and CK5/6 were positively correlated ($R^2 = 0.22$). There was no significant association between staining pattern and race, BMI or age at diagnosis. Double positive tumors were equally common among African-American (39%) and other patients (40%).

Conclusions: We conclude that the association between the Tneg breast cancer phenotype with race and BMI also correlates with CK5/6 and EGFR expression in these tumors. Expression of EGFR and CK5/6 in Tneg tumors is independent of patient race or BMI at presentation.

160 Effect Polysomy of Chromosome 17 on the Evaluation of HER-2/neu Status in Breast Cancer

K Lee, CL Hyun, JH Chung, G Choi, SY Park. Seoul National University College of Medicine, Seoul, Korea; Seoul National University Bundang Hospital, Seongnam, Gyeonggi, Korea.

Background: Accurate assessment of HER-2 status is important for the management and prognostication of patients with breast cancer. Polysomy of chromosome 17 is frequently observed in assessing HER-2 gene status and its influence on HER-2 gene and protein expression remains controversial. The aim of the study was to investigate the effect of polysomy 17 on the evaluation of HER-2 status by immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and chromogenic in situ hybridization (CISH).

Design: We performed dual-probe FISH for the simultaneous enumeration of the HER-2 gene and the centromeric region of chromosome 17 and single-probe CISH for the detection of HER-2 gene amplification, as well as IHC to detect HER-2 protein overexpression, on 309 consecutively resected invasive breast cancers.

Results: Polysomy 17 was detected in 32.0% (99/309) of cases; 12.3% (18/146) of IHC 0 or 1+, 42.8% (48/113) of IHC 2+ and 66.0% (33/50) of IHC 3+ cases (P<0.001). Sixty cases (19.4%) composed of 15 IHC 2+ and 45 IHC 3+ cases showed amplification by FISH. Polysomy 17 was more frequent in FISH-positive cases than in FISH-negative cases (65.0% vs. 24.1%; P<0.001). In addition, IHC 2+ or 3+ /FISH-negative cases showed significantly higher rate of polysomy 17 than IHC 0 or 1+ cases (40.8% vs. 12.3%; P<0.001). Fifty eight cases (18.8%) showed amplification by CISH. Overall, the concordance rate of FISH and CISH was 96.8%. Among the ten discordant cases, five cases with disomy 17 showed low level amplification by FISH and borderline copy numbers (3-5 dots/nucleus) by CISH. One case revealed monosomy 17 and showed low level amplification by FISH but not by CISH. Four cases had high polysomy 17 and showed amplification by CISH but not by FISH.

Conclusions: Increase in HER-2 gene copy numbers according to polysomy 17 may lead to HER-2 protein overexpression in a subset of breast cancer, even in the absence of gene amplification. The high level of concordance between FISH and CISH suggests that, in general, CISH can be an alternative to FISH for determination of HER-2 gene amplification. However, cases with borderline copy numbers or amplification of HER-2 gene by CISH may need a determination of the chromosome 17 status to rule out polysomy 17.

161 Adenoid Cystic Carcinoma of the Breast: Frequent Margin Involvement after Primary Surgery

A Lytwyn, N Hodgson, D Hileeto, S Bacopoulus, D Browing, N Howatt, LJ Elavathil. McMaster University, Hamilton, ON, Canada.

Background: Adenoid cystic carcinoma of the breast (ACCB) is a rare neoplasm with low risk of regional and distant metastases, and only exceptionally causes death. Although ACCB is described as generally having well circumscribed margins, we encountered a patient who needed repeated re-excisions to obtain clear margins. We therefore reviewed our experience with ACCB to identify whether ACCB is more locally extensive than is clinically or radiographically appreciated.

Design: We searched the Pathology database from 1987-2006 for ACCB. 2 pathologists independently reviewed the slides; discrepancies were resolved by consensus review. Immunohistochemistry was performed on representative blocks.

Results: Database search identified 13 reports of ACCB. We retrieved slides from 11 cases and pathology review confirmed ACCB. Blocks from 10 cases were available for immunohistochemistry. Median patient age was 57 years (range 23-82). 5/8 patients (63%) presented with a palpable lump and 4 (50%) had an abnormal mammogram. Median tumor size was 3.0cm (range 1.4-7.0). 2 tumors were grade 3 by Ro criteria; the remainder were grade 2. 9 were negative for estrogen and progesterone receptors; 1 showed positive staining for both receptors. All were positive for S-100, smooth muscle myosin heavy chain, or p63; all showed positive staining for c-kit and were negative for Her2neu. 7/11 (64%) patients underwent lumpectomy as initial surgery. 5 (71%) required re-excision for margin involvement. 1/3 patients who underwent mastectomy as primary surgery had a positive margin. 1 patient had a diffusely infiltrating tumor in the resection that was initially reported as negative. Indeed in this case it was difficult to distinguish neoplastic tubules from normal ducts; lack of estrogen staining was helpful to establish the full extent of neoplastic infiltration.

Conclusions: The potentially locally extensive nature of ABBC has not been reported in the literature to our knowledge. Neither clinical nor mammographic examination delineated full tumor extent. The pathologist needs to be aware of this when examining the breast specimen. Lack of estrogen staining may help delineate the full extent of the tumor. Future use of MRI in pre-operative assessment may prevent high positive margin rate when lumpectomy is planned.

162 Correlation of *HER2* Gene and Chromosome 17 Status with Pathologic Factors in Her2 2+ Invasive Breast Cancer

G MacGrogan, F Chibon, I de Mascarel, G Sierankowski, V Brouste. Institute Bergonié, Bordeaux, France.

Background: Most Her2 testing guidelines recommend that cases scoring Her2 2+ by immunohistochemistry (IHC) should be analysed by FISH to determine *HER2* gene status in order to confirm eligibility for Trastuzumab therapy in breast cancer. The aim of our study was to determine *HER2* gene and Chromosome 17 (CEN-17) status in a series of IHC Her2 2+ cases and study the relationship between pathological characteristics of the tumors and Her2 gene amplification.

Design: 108 consecutive cases of Her2 IHC 2+ invasive breast cancers were tested by FISH using the Dako *HER2* FISH pharmDx™ kit. The Her2 IHC protocol was performed after antigen retrieval (citrate buffer pH6) using the polyclonal AO485 antibody (Dako) diluted to 1/1500. The FISH assay included the hybridization of a Texas Red-labelled HER2 DNA probe and a fluorescein-labelled peptide nucleic acid probe targeted to the centromeric region of chromosome 17 (CEN-17). *HER2* gene copy number, CEN-17 copy number and *HER2*/CEN-17 ratio were determined and tumors with a *HER2*/CEN-17 ratio ≥ 2 were considered *HER2* amplified. The relationship between *HER2* and CEN-17 status anhd tumor SBR grade, mitotic count, estrogen receptor (ER), progesterone receptor (PR) status and percentage of IHC Her2 positive cells was studied.

Results: A *HER2*/CEN-17 ratio ≥ 2 and a *HER2* gene copy number > 4 were observed in 36(33.3%) and 49 (45.4%) cases, respectively. Chromosome 17 polysomy (CEN-17 >2.25) was observed in 39 (36.1%) tumors. Using the Chi-square test, no significant correlation was found between chromosome 17 polysomic status and the different pathological factors. Significant positive correlations were found between *HER2*/CEN-17 ratio ≥ 2 and IHC Her2 >60% ($p=1.1.10^{-5}$), SBR grade (p=0.0001), nuclear atypia (p=0.03) and mitotic count (p=0.008). No correlation was found between ER, PR and *HER2*/CEN-17 ratio ≥ 2. By multivariate analysis, IHC Her2>60% [OR=11.9, 95%CI(3- 44);$p=2.10^{-7}$] and SBR grade3 [OR=6.2, 95%CI(2.3 -16.7);$p=3.10^{-7}$] were independent factors predicting *HER2* amplification status. All SBR grade3 cases with more than 60% IHC Her2-positive cells had a *HER2*/CEN-17 ratio ≥ 2.

Conclusions: In our series of consecutive Her2 IHC 2+ cases, one third demonstrated *HER2* amplification using the *HER2*/CEN-17 ratio ≥ 2 criterion and one third had chromosome 17 polysomy. Pathological factors, in particular SBR grade3 and more than 60% IHC Her2-positive cells, were significantly correlated with *HER2* gene amplification.

163 CK5 Is More Sensitive Than CK5/6 in Identifying Basal-Like Mammary Duct Carcinoma (BL-MDC) by Immunohistochemistry (IHC)

L Marinescu, W He, RW Cartun, D Stevens, A Ricci, Jr. Hartford Hospital, Hartford, CT.

Background: A working diagnosis of basal-like mammary duct carcinoma (BL-MDC) may be inferred for high-grade, ER/PR/HER2 negative breast tumors if CK5/6 and/or epidermal growth factor receptor (EGFr) are positive by IHC assay. In this study we compared the sensitivity and specificity of monoclonal antibodies (mAb) CK5 and CK5/6 in characterizing potential examples of BL-MDC.

Design: As part of a separate companion study of atypical medullary carcinoma, 16 cases of high-grade and "triple-negative" MDC were collected from the Hartford Hospital Surgical Pathology files for the years 1999-2005. All cases were probed with mAb to CK5 (clone XM26), CK5/6 (clone D5/16B4) and EGFr (clone 31G7). CK reactivities were evaluated by a summed score (ss) of intensity (0+ none, 1+ faint, 2+ distinct or 3+ intense) and distribution (none "0", isolated cells, up to 1/100 "1", cell clusters, 1/100 – 1/10 "2", focal 1/10 – 1/3 "3", patchy 1/3 – 2/3 "4" or diffuse, over 2/3 "5") after the San Antonio/Allred system (Modern Pathol 1998). EGFr was scored 0 to 3+ analogous to routine HER-2/neu IHC assays.

Table 1

Case number	ss CK5	ss CK5/6	EGFr
#1	6.5	3	3
#2	0	0	0
#3	7	5.5	2
#4	8	6.5	2
#5	7	6	3
#6	7	5	0
#7	7.5	6	0
#8	7.5	6	1
#9	3	2	0
#10	2	0	0
#11	7	6	3
#12	0	0	3
#13	3	0	2
#14	6.5	5	2
#15	7	6	1
#16	3.5	3	1

Results: By the criterion of either CK5/6 or EGFr positivity, 14/16 of these high-grade and triple-negative tumors could be classified as BL-MDC. In each of these cases the CK5 outperformed the CK5/6 mAb according the summed score combining intensity and distribution of immunoreactivity (average ssCK5 = 5.16 versus ssCK5/6 = 3.75). One of the remaining two CK5/6 & EGFr negative cases was positive with CK5 (#10).

Conclusions: (1) There is a high concordance between CD5 & CK5/6, 15 of 16 cases yielding similar results. (2) Higher ssCK5 as compared with ssCK5/6 is consistent with a greater sensitivity of the former and results in easier IHC interpretation in this application. (3) Whether the one discordant case (#10) reflects greater sensitivity (versus lower specificity) remains an open question.

164 Lymphangiogenesis and Angiogenesis in Breast Cancer: Correlation with Clinicopathologic Features and Axillary Lymph Node Metastases

VFZ Marinho, K Metze, GFS Rocha, FSF Sanches, H Gobbi. Universidade Federal de Minas Gerais & UFMG, Belo Horizonte, Minas Gerais, Brazil; Universidade Estadual de Campinas & UNICAMP, Campinas, São Paulo, Brazil.

Background: Previously the study of lymphatic vessels and lymphangiogenesis has been limited by a lack of specific markers. Recently however, the new D2-40 antibody, which specifically marks lymphatic vessels, was released. In this study we evaluated lymphangiogenesis and angiogenesis in invasive mammary carcinomas (IMC), the presence of lymphatic (LVI) and blood vessel (BVI) invasion and the relationship of LVI and BVI with lymph node axillary metastases.

Design: We studied 123 cases of IMC stratified in 3 subgroups: macrometastases (Mac-Met), micrometastases (Mic-Met), and lymph node negative (LN). Intratumoral and peritumoral lymphatic (LMVD) and blood (BMVD) microvessel density were evaluated by immunohistochemistry (IHC) using the D2-40 and CD31 antibodies. LVI and BVI were assessed in H&E and IHC-stained sections. LVI, BVI, LMVD, and BMVD were related to histologic tumor type and grade, estrogen (ER) and progesterone (PR) receptors, E-cadherin, Ki67, p53, and Her2/*neu* expression of primary tumors. Intratumoral and peritumoral LMVD and BMVD were counted separately, in areas of invasive tumor with the greatest number of distinctly vessels ("hot spot"). After the highest LMVD and BMVD areas were identified, single lymphatic and blood vessels were visually counted using a X200 (0.74 mm^2) magnification, in 5 consecutive fields (total area = 3.69 mm^2).

Results: IHC was superior in detecting both LVI and BVI when compared to H&E (specificity for LVI= 96.6%; specifitity for BVI= 98.1%). LVI and BVI were positively related to histologic grade, Ki67 index, and Her2/*neu* expression, and inversely related to ER and PR ($p<0.05$). When considering all 123 cases, or when cases were stratified in subgroups, peritumoral BMVD and intratumoral BMVD were both superior to peritumoral LMVD and intratumoral LMVD respectively ($p<0.0001$). Intratumoral LMVD was scanty and inferior to peritumoral LMVD ($p<0.0001$).

Conclusions: In conclusion, although angiogenesis parallels the progression of breast lesions, lymphangiogenesis appears to be lacking, resulting in complete or partial absence of intratumoral lymph vessels. Supports: Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq).

165 Is Basal Cytokeratin (CK) or Epidermal Growth Factor Receptor (EGFR) Expression Predictive of BRCA1 Mutation Status in Women with Triple Negative Breast Cancers?

A Martyniak, MJ Kandel, Z Stadler, S Masciari, L Harris, A Miron, A Richardson, SJ Schnitt, JE Garber, LC Collins. Beth Israel Deaconess Medical Center, Boston; Dana Farber Cancer Institute, Boston; Brigham and Women's Hospital, Boston; Harvard Medical School, Boston, MA.

Background: Most breast cancers that are ER, PR and HER2 negative ("triple negative") are "basal-like" by DNA microarray and immunophenotypic analysis, and 80% of breast cancers in women with germline *BRCA1* mutations are triple negative. Whether expression of biomarkers characteristic of basal-like breast cancers helps to define a subset of women with triple negative breast cancers who are likely to harbour *BRCA1* mutations is an unresolved issue.

Design: We randomly identified 148 women from the Dana-Farber/Harvard Cancer Center SPORE annotated specimen bank with primary invasive, triple negative breast cancers as determined by routine clinical ER, PR and HER2 assays. Tissue microarrays (TMAs) were constructed by obtaining triplicate 0.6mm cores from paraffin blocks. In 35 cases, blocks were not available for TMA construction, but unstained slides were obtained. Slides cut from the TMAs and the unstained slides were immunostained for ER, PR, and HER2 (to confirm triple negative status) and for several markers useful in defining the basal-like phenotype, including three basal CKs (CK5/6, CK14, and CK17) and EGFR. Full sequencing analysis for *BRCA1* germline mutations was performed on DNA from blood in all cases. The final study population consisted of 144 cases in which triple negative status was confirmed and there was sufficient material for analysis of basal cytokeratins and EGFR.

Results: One or more basal CKs were expressed in 97 cancers (67%) and 102 (71%) showed EGFR expression. *BRCA1* germline mutations were found in 20 women (14%). The frequency of expression of basal CK and EGFR according to *BRCA1* mutation status is shown in the Table:

	BRCA1 pos (n=20)	BRCA1 neg (#pos/#evaluable)	p-value
Basal CK+ (one or more)	13 (65%)	84/124 (68%)	NS
EGFR+	15 (75%)	87/122 (71%)	NS
Either Basal CK or EGFR+	19 (95%)	107/123 (87%)	NS

Conclusions: Basal CK and EGFR expression are both highly prevalent among triple negative breast cancers. The frequency of expression of basal CK and EGFR was similar in women with and without *BRCA1* mutations. Therefore, basal CK and/or EGFR expression cannot be used to help predict which women with triple negative breast cancers are likely to harbour *BRCA1* germline mutations.

166 Association between Lobular Neoplasia and Columnar-Cell Lesions in Breast Needle Core Biopsies Performed for Calcifications

SE Mendrinos, BP Wu, CM Carman, SI Fisher, JL Connolly. Sentara Norfolk General Hospital, Norfolk, VA; Breast Care Specialists/Sentara Norfolk General Hospital, Norfolk, VA; Beth Israel Deaconess Medical Center, Boston, MA.

Background: The diagnosis of lobular neoplasia (LN), including atypical lobular hyperplasia (ALH) and lobular carcinoma in-situ (LCIS), on needle core biopsy (NCB) for calcifications is most often an incidental finding but carries significant clinical implications. LN is considered a risk factor for subsequent development of carcinoma in either breast. The purpose of this study is to describe any associated pathologic findings (APF) found with LN on NCB's performed on patients with only mammographic calcifications, as well as to ascertain if the risks carried by LN are augmented by these APF.

Design: From June 2005 through August 2006, close to 1265 NCB's were performed at Sentara Norfolk General Hospital. Of these, 748 were performed for mammographic calcifications only; out of this subset, 20 had ALH/LCIS as the primary diagnosis (2.67%). Patients with additional atypical pathologic, radiologic or clinical findings were not included. The pathologic features were reviewed retrospectively by two separate breast pathologists. Clinical followup was available for all 20 patients.

Results: All NCBs were obtained by mammotome (14-gauge, 4 cases; 11-gauge, 8 cases; 8-gauge, 6 cases). APF included columnar cell change (CCC) and columnar cell hyperplasia without atypia (CCH) in 15/20 cases (75%), usual ductal hyperplasia in 6 cases (30%), adenosis and sclerosing adenosis in 3 cases (15%), and fibroadenomatous change in 2 cases (10%). Within a followup period up to 15 months, one patient developed ipsilateral low-grade infiltrating ductal carcinoma. This patient had LCIS with CCC on her NCB.

Conclusions: Our study demonstrates that ALH/LCIS on NCB shows a frequent association with CCC/CCH, lesions that are associated with mammographic calcifications. In addition to this histologic relationship, both lesions share a common immunophenotypic profile with loss of immunoreactivity for cytokeratin 5/6 and strong expression of estrogen and progesterone receptors. While our followup period is relatively brief, one patient with LCIS and CCC developed ipsilateral well-differentiated ductal carcinoma. Additional followup and study is required to determine if CCC/CCH confers increased risk of ipsilateral breast carcinoma in addition to the bilateral risk posed by the associated ALH/LCIS.

167 Expression of ERα and ERβ in Lobular Carcinoma In Situ

LP Middleton, GH Perkins, SL Tucker, AA Sahin, SE Singletary. UT MD Anderson Cancer Center, Houston, TX.

Background: Estrogen is a modulator of cell growth and differentiation. It mediates most of its function through members of the estrogen receptor (ER) family; ERα and ERβ. Accordingly, estrogen receptor binding modulators have become a prevention strategy in oncogenesis. Tamoxifen is a proven anti-estrogen agent that reduces the risk of invasive breast cancer by more than 50% in high risk women. The presence of lobular carcinoma in situ (LCIS) is a known risk factor for the development of breast cancer; however, the use of tamoxifen for prevention is based upon data for ER-positive (ERα) LCIS associated with invasive carcinoma, but limited data exist on the use of tamoxifen in cases of ER-positive (ERα) LCIS occurring in the absence of invasion. We examined ERα and ERβ in cells of patients with LCIS to determine the relationship of ERα to ERβ, to determine whether it is of clinical value to measure ERβ along with ERα in patients with LCIS and to investigate whether ERβ can be a novel target in therapy.

Design: We retrospectively examined core biopsy material from 50 patients diagnosed with LCIS. Histology was reviewed. Coexisting pathology was noted. E-cadherin, ERα, and ERβ immunohistochemical staining was performed. The degree of ERα and ERβ nuclear reactivity was quantified.

Results: Patients' mean age was 55 years (range 37-79 years). The mean follow-up duration was 48 months. The nuclear grade of the tumors was 1 in 48 cases and 2 in 2 cases. Coexisting pathologic findings included columnar cell alterations in 30% of cases and fibrocystic changes in 25%. All LCIS was E-cadherin negative. All cases were ERα and ERβ positive. The staining intensity of ERβ was strong and included staining of periductal stromal cells. The median percentage of cells staining for ERα was 75% and ERβ was 70% (range, 10% weak positive to 100% strong positive). There was a statistically significant relationship between ERβ staining intensity and incidence of ipsilateral breast cancer ($p=0.010$).

Conclusions: The presence and intensity of both stromal and glandular ERβ immunoreactivity suggests that the action of estrogen on LCIS is on both the stromal and glandular cells. Future studies are needed to determine whether the presence of ERβ in LCIS could be targeted to influence the treatment of this disease and perhaps alter the natural history of this entity.

168 Conserved Patterns of Copy Number Aberrations (CNAs) Detected with Array Comparative Genomic Hybridization (aCGH) Characterize & Distinguish *In Situ* Lobular & *In Situ* Ductal Carcinoma

C Mies, M Guttman, K Dudycz-Sulicz, D Baldwin, G Grant. University of Pennsylvania, Philadelphia, PA.

Background: Gains & losses of DNA, a.k.a. copy number aberrations (**CNAs**), are an apparent product of genomic instability & are characteristic of human cancer. Conserved patterns of CNAs within different types of cancer are the presumed basis of phenotypic differences & are inferred to impart a selection advantage to the cells that harbor them. Currently, pathologists use *morphological* differences to divide mammary carcinoma *in situ* (CIS) into two major subtypes – lobular (**LCIS**) & ductal (**DCIS**) – both putative non-obligate precursors of invasive carcinoma. The purpose of this study was to 1) identify conserved patterns of CNAs that characterize the morphologically-distinct ductal & lobular subtypes of mammary CIS; and 2) determine whether there are genomic regions that are linked to each phenotype.

Design: Because human mammary CIS can be reliably diagnosed only in histologic sections of fixed, paraffin embedded tissues, we adapted our experimental & analytic procedures to this venue. We DOP-PCR amplified DNA isolated from UV-laser capture microdissected cancer cells of different types & hybridized it to a 6,912 BAC clone microarray. We profiled DCIS (N=25) & LCIS (N = 36) & analyzed the arrays using our Multiple Sample Algorithm approach, which provides multiple sample significance for each region of the genome across a cohort of samples. We then conducted a differential search to determine significantly different aberrations between the DCIS & LCIS cohorts.

Results: In **LCIS**, we found 1,061 regions of significantly conserved gain or loss at the p<.05 level; 650 regions were significantly aberrant at the p<.01 level. In **DCIS**, we found 880 regions of significantly conserved gain or loss at the p<.05 level; 489 were significant at the p<.01 level. Comparing the two, we found 352 regions of differential CNA between the LCIS & DCIS groups; of these, 191 were significantly more frequent in LCIS & 160 were more frequent in DCIS.
Conclusions: 1) There are patterns of conserved CNAs detected by high-resolution aCGH that are intrinsic to the morphologically-defined LCIS & DCIS subtypes of mammary carcinoma; and 2) there are regions of the genome that are differentially affected in LCIS *vs* DCIS.

169 Expression of Estrogen Receptor Co-Activator AIB1 in Breast Cancer

A Morimiya, D Turbin, D Hunstman, Q Zhang, M Jeng, H Nakshatri, S Badve. Indiana University, Indianapolis, IN; University of British Columbia, Vancouver, BC, Canada.
Background: The functional activity of Estrogen receptor (ER) is significantly modified by a number of intracellular proteins collectively termed co-activators or co-repressors. Amplified in breast cancer -1 (AIB-1) is one of the co-activators that has been purported to play an important role in breast cancer. In addition, AIB1 can function in a hormone independent manner and convey growth factor and proliferation signals to ER and enhance transcription of STATs and NFκB. To date, it is the only p160 co-activator implicated in cancer, and has been suggested to be an oncogene. Overexpression of AIB1 in transgenic mice leads to increased mammory tumor incidence and elevated serum levels of growth factor IGF-1. In this study we analyze the expression of this protein in breast cancer and evaluate its prognostic value.
Design: Expression of AIB1 was analyzed in tissue microarray sections from a series of over 400 well characterized breast cancers with 20-year survival data. Immunohistochemical staining was performed with a monoclonal anti-AIB1 antibody using non-biotin based polymer detection system and DAB as the chromogen. A modified Histoscore system was used to analyze both the intensity and percentage of staining (Score= I x P). Cutoff point for positivity was determined using the X-tile program and statistical analysis performed using the SPSS software package.
Results: Nuclear expression of AIB1 was predominantly in seen in cancer cells, with minimal or no staining in benign cells. A large number of cancers showed weak nuclear expression in majority of tumor cells. High level expression (>14) was seen in 146 of the 395 evaluable cores. This was negatively associated with ER status (p= 0.0001), PR status (p< 0.00001), and positively with Her-2 (p=0.0005), p53 (p=0.007) and increased Ki-67 expression (p= 0.003). High level expression did not predict for poor survival (p=0.262).
Conclusions: The strong association with growth factor signaling pathway and negative relationship with ER pathway seem to confirm suggest that AIB1 in breast cancer plays a role independent of ER, probably by enhancing transcription of NFκB. These findings support the hypothesis that AIB1 is an oncogene.

170 Atypical Ductal Hyperplasia at Margin of Breast Biopsy – Is Re-Excision Indicated?

C Moung, S Arora, T Menes, IJ Bleiweiss, C Nagi, S Jaffer. The Mount Sinai Medical Center, New York, NY; City Hospital at Elmhurst, Elmhurst, NY.
Background: The diagnosis of atypical duct hyperplasia (ADH) on core needle biopsy warrants an excision because of a high frequency of associated intraductal carcinoma (DCIS). The presence of DCIS at or close to (within 1mm) a margin also warrants excision to ensure negative margins. Logically then the presence of ADH at or close to a margin should also warrant an excision to ensure negative margins. However, controversy exists regarding the need to re-excise ADH involving a margin. The purpose of this study was to determine the rate of residual pathology in patients that underwent re-excision for ADH involving a margin.
Design: A retrospective review of the pathology database from 1/1/2000 to 6/1/2006 identified 44 out of 870 lumpectomy specimens with ADH involving the margin. Initial lumpectomy and subsequent re-excision slides were reviewed to verify and analyze the diagnosis of ADH near the margin for the presence of residual disease associated with the biopsy cavity.
Results: The indication for lumpectomy was either a mammographic (37,84%) or physical (7,16%) finding. These patients had ADH alone (33, 75%), ADH and DCIS (9, 20%) or ADH with invasive carcinoma (2, 5%). The morphology of the ADH was mostly cribriform with admixed micopapillary (6 cases) and columnar features (2). The ADH was either present unifocally or as scattered foci at the margin. The decision to re-excise was based on the surgeon's preference such that only 24 patients (55%) underwent a re-excision, 13 were not excised and 7 were lost to follow up. Residual ADH or cancer was found in 14 of 24 patients (58%). In patients with ADH alone (n=15), 6 (40%) had residual pathology: ADH (2), DCIS (2) and invasive carcinoma (2). In this group 27% of patients were upgraded to DCIS or invasive carcinoma. Six of the 7 (85.7%) patients with DCIS had residual disease in the form of ADH (3) or DCIS (3). In the 2 patients who had invasive carcinoma, both had residual ADH.
Conclusions: ADH found at the margin of a lumpectomy specimen is associated with a high rate of residual ADH and cancer. Although no correlation with the pattern of ADH was found, the presence of scattered ADH was predictive of residual disease. Over a quarter of patients with initial diagnosis of ADH were upgraded to DCIS or invasive carcinoma. Furthermore patients with DCIS or invasive carcinoma had a high rate (89%) of residual ADH or carcinoma. Re-excision in all patients with ADH involving the margin is recommended.

171 Fibrosclerotic Lesions in Phyllodes Tumors May Give Rise to Metaplastic Tumors

JS Mueller, FI Boulos, DL Page. University of Chicago, Chicago, IL; Vanderbilt University, Nashville, TN.
Background: Fibrosclerotic lesions with squamous morules have been described in breast lesions such as papillomas, complex sclerosing lesions and nipple adenomas. These lesions are believed to be premalignant and may give rise to metaplastic tumors of the breast (Gobbi et al, Mod Pathol 2003). Occasional areas of fibrosclerosis with squamous morules have also been noted within Phyllodes tumors but have not yet been described. The aim of this study was to review previous cases of Phyllodes tumors and determine if these fibrosclerotic lesions were present and to further characterize associated changes. We postulate that these lesions may also be the genesis of metaplastic tumors as well as mixed malignancies reported in Phyllodes tumors.
Design: Approximately 150 Phyllodes tumors with multiple available microscopic slides were reviewed from a series of >1000 received as referral cases in the Breast Pathology Consult Service at Vanderbilt University from 1985-present. Hemotoxylin and eosin-stained slides were reviewed as well as available immunohistochemical stains. Of the 150 cases, eight were found to have areas meeting histologic criteria for these characteristic fibrosclerotic lesions. These cases were further studied and the lesions were described as was any other significant pathology such as associated metaplastic tumor or carcinoma.
Results: Of the cases reviewed, eight had histologically identifible areas of fibrosclerosis with squamous morules. Three were benign, three were borderline and two were malignant Phyllodes tumors. Two of the borderline Phyllodes tumors had associated metaplastic tumors with fibromatosis-like phenotype. One of the malignant Phyllodes had a liposarcomatous stroma and the other had a fibrosarcomatous stroma. One of the three benign Phyllodes had an associated infiltrating low-grade mammary carcinoma. Immunohistochemical stains reviewed on several cases showed the spindle cells within the fibrosclerotic foci to be positive for high molecular weight cytokeratins (orthokeratin and CK903).
Conclusions: Cytokeratin positive fibrosclerotic foci with squamous morules have previously been described in sclerotic lesions associated with metaplastic breast tumors (CSL, papillomas etc). These probable nascent premalignant lesions are now identified in Phyllodes tumor - adding to those lesions which have already been described. These lesions may underly the genesis of metaplastic tumors and mixed malignancies in Phyllodes tumors.

172 Validation of Tissue Microarray Analysis in a Large Prospectively Accrued Patient Cohort of Lymph Node Negative Breast Cancer

AM Mulligan, D Pinnaduwage, SB Bull, FP O'Malley, IL Andrulis. Mount Sinai Hospital, Toronto, ON, Canada; Toronto, ON, Canada.
Background: Gene expression profiling has generated vast data on candidate genes involved in key molecular events in carcinogenesis. Subsequent validation of the clinical importance of such genes necessitates large scale analyses of tumor tissue.
Design: A consecutive series of patients (n=1,902) with lymph node negative breast cancer was prospectively accrued from eight Toronto hospitals from 1987 to 1999. Fresh tissue was obtained on 977 of these tumors for molecular analysis and paraffin-embedded tumor blocks were obtained on 888 tumors for construction of tissue microarrays (TMAs). ER and PgR status was determined by ligand binding assay (LBA) or immunohistochemical (IHC) methods, depending on laboratory practice at the time of surgery. Our initial studies involved assessment of single genes (HER2 amplification and p53 mutation status). Results from these studies showed a significant risk of recurrence in patients with HER2 amplified tumors (HR 1.68, CI=1.07-2.63, p= 0.02) and p53 mutations (HR 1.69, CI 1.10-2.58, p= 0.02). We are currently performing gene expression array analysis on a cohort of the frozen tumor samples. In order to ensure that the cohort of tumors within the TMA are representative of the entire cohort, we assessed the expression of ER, PgR, p53 and HER2 by IHC and assessed correlations with outcome using Kaplain-Meier curves, log-rank significance testing and in multivariate analysis using Cox proportional hazards models.
Results: ER/PgR assessment by IHC on the TMAs significantly correlated with ER/PgR as detemined in the original cohort (Kappa=0.71, CI=0.65-0.77, p<0.0001 and Kappa=0.59, CI=0.52-0.65, p<0.0001). In line with earlier molecular studies in this cohort, a significant risk of recurrence was found in patients with HER2 IHC positive tumors (HR 2.29, CI=1.34-3.94, p=0.002) and p53 overexpressing tumors (HR 1.81, CI=1.19-2.75, p=0.005) in Cox univariate analysis.
Conclusions: We have shown that TMAs constructed from a large, prospectively accrued patient cohort of LNN breast cancer are representative of the cohort. We will now use these TMAs to validate putative candidate genes that are identified from our ongoing gene expression array analysis. This validation study paves the way for the parallel investigation of candidate genes, thus accelerating the transition from basic research to clinical application.

173 Using Morphology and Phenotype To Predict BRCA1 Mutation Carrier Status in Breast Cancer

AM Mulligan, N Weerasooriya, IL Andrulis, FP O'Malley. Mount Sinai Hospital, Toronto, ON, Canada; Toronto, ON, Canada.
Background: The basal phenotype has been shown to be disproportionately represented in breast cancers from BRCA1 mutation carriers. Our aim in this large series of patients with BRCA1 associated breast cancers, was to use an extended panel of biomarkers to further characterize tumors in BRCA1 mutation carriers.
Design: Tissue microarrays (TMAs) were constructed from tumors from 59 BRCA1 carriers and 57 control patients 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 carriers and controls and scored using Allreds method.

Results: Tumors from BRCA1 mutation carriers were higher grade (Odds ratio (OR) 42.7), showed a marked lymphocytic infiltrate (OR 5.50) and a syncytial growth pattern (OR 5.50). BRCA1-associated tumors were ER/PgR/HER2 negative and CK5 positive, in keeping with a basal phenotype. In addition, these tumors were more frequently p53 positive, had a high MIB-1 index and were negative for CK8/18, cyclin D1 and p27 (Table 1).
Conclusions: We have confirmed, in a large series of patients with known BRCA1 mutations, that these tumors cluster with the basal phenotype. Additionally, we have demonstrated that they have a high MIB-1 index, are p53 postive, but lack p27, cyclin D1 and CK8/18 expression. The combination of these specific morphologic and phenotypic features could be used to more specifically target genetic testing in the appropriate clinical setting.

Table 1

Biomarker	BRCA1 mutation carriers N(%)	Non-carriers N(%)	Odds Ratio	Chi-square (p-value)
ER	9(15)	29(51)	0.16	17.5 (0.0002)
PR	4(7)	19(33)	0.13	15.7 (0.0004)
p53	31(53)	10(18)	5.68	15.5 (0.0004)
CK8/18	12(20)	30(53)	0.18	13.7 (0.001)
CK5	34(58)	4(7)	17.35	24.9 (<0.0001)
Cyclin D1	2(3)	6(11)	0.23	5.3 (0.07)
MIB-1	13(22)	1(2)	18.38	8.0 (0.02)
CK14	9(15)	3(5)	3.46	4.7 (0.09)
p27	1(2)	14(25)	0.06	8.3 (0.02)
Vimentin	4(7)	0(0)	5.64	1.7 (0.43)
HER2	1(2)	2(4)	0.45	0.4 (0.81)

Odds ratio of biomarker expression in BRCA1 mutation carriers vs.controls. N(%)=number and percentage of patients with positive expression of biomarker

174 Perpendicular Margin (PM) and Shaved Margin (SM) Evaluation by Pathologists Versus Separate Shaved Cavity Margin (SCM) by a Breast Surgeon: Which Is More Accurate and Efficient?

M Murray, A Barrio, S Patil, E Brogi, T Nehhozina, A Heerdt, LK Tan. Memorial Sloan-Kettering Cancer Center, NY, NY.
Background: There is no uniform technique for margin assessment. PM enables measurement of tumor distance to margin, but is time-consuming for pathologists. SM allows for evaluation of a greater marginal surface, but cannot distinguish close from true positive margin. In SCM the cavity margins are shaved and submitted by the surgeon. Our aim was to study the efficacy of these 3 techniques.
Design: In 2004, our protocol for margin evaluation was changed from a PM (Group A) to a SM technique (Group B), and recently one breast surgeon adopted the SCM method (Group C). From 6/2003 to 6/2006, pathology records for this surgeon were reviewed for cases of primary excision (EX) performed for biopsy-proven invasive carcinoma (INV) or ductal carcinoma in situ (DCIS). In Groups A and B, the EX was oriented by the surgeon but the margins were, respectively, differentially inked or shaved by the pathologists. In Group C, the surgeon submitted separate cavity margins that were pathologically processed as shaved margins. Close margin in PM was defined as <2 mm. Differences between the groups were examined using the ANOVA for continuous variables and Pearsons Chi-square test for categorical variables.
Results: The groups were similar with respect to age, tumor size, type and grade, extent of DCIS, vascular invasion, and multifocality. The number of positive and/or close margins were similar in Groups A and B (72% and 71%), as were the re-excision rates (48%). Group C had a significant decrease in the number of positive margins (42%, p= <.001), re-excision rate (32%, p=.03) and had the highest number of cases with residual disease in the re-excisions (50%, versus 40% and 27% in Groups A and C). The difference in residual disease between Groups A and C was not statistically significant, but the time spent by pathologists in margin evaluation was reduced in Group C. DCIS was the main finding in the re-excision specimens in all groups.
Conclusions: SCM is the best method in our study because 1) it decreases the re-excision rate; 2) it results in better detection of residual tumor in the re-excisions; 3) it reduces the time pathologists spend in margin evaluation.

	Group A N=100	Group B N=100	Group C N=100	p value
Margin +	32	71	42	<.001
Margin close*	40	NA	NA	
Re-EX	48	48	32	.03
RE-EX DCIS	15	7	14	.10
RE-EX INV	4	6	2	

* For analysis, close margins were included in the positive margin category

175 Primary Angiosarcoma of the Breast: Clinicopathologic Analysis of 45 Cases

AF Nascimento, CP Raut, CDM Fletcher. Brigham and Women's Hospital & Harvard Medical School, Boston, MA.
Background: Mammary angiosarcoma is a rare neoplasm, accounting for about 0.05% of all primary malignancies of the breast. It is currently believed that histologic grading of mammary angiosarcomas plays an important role in prognostication.
Design: Forty-five cases of primary angiosarcoma of the breast were retrieved from our files. H&E-stained sections were available for review in 44 cases and diagnoses were confirmed. In one case only slides from subsequent metastases could be re-reviewed. Clinical details and follow-up information were obtained from referring pathologists and clinicians, and by chart review. All statistics were performed using Fisher exact test and only $p < 0.05$ was considered significant.
Results: All patients were female with ages ranging from 15 to 74 years (median 41). All tumors examined were located within breast parenchyma. The right side (62.5%) was more commonly affected than the left side (32.5%). Tumor was bilateral in two cases. Tumor size varied from 0.7 to 25 cm (median 5). Most patients presented with a palpable, painless mass. One patient had a history of radiation for treatment of breast carcinoma. Histologically, tumors were graded using Rosen's 3-tier system: 15 tumors (34.1%) as low grade, 16 (36.4%) as intermediate grade and 13 (29.5%) as high grade. Thirty-two patients were treated surgically, 10 underwent chemotherapy and 11 patients received radiotherapy. Follow-up was available in 35 patients (78%). Nine patients (25.7%) showed evidence of local recurrence within 12 to 38 months (median 24) after diagnosis. Eighteen patients (51.4%) have developed metastases to skin, lung, liver, pericardium, ovaries, bones and lymph node. Time interval between diagnosis and metastasis ranged from 9 to 108 months (median 23.5). Seventeen patients (48.6%) have died of disease and one died of disseminated breast carcinoma. Two patients are alive with disease and 14 patients are alive with no evidence of disease. Statistical analysis evaluating correlation between tumor grade and rate of local recurrence, metastasis, and death due to disease showed no significant difference among tumors of different grades.
Conclusions: Mammary angiosarcoma is a rare disease that affects relatively young patients. Although this tumor appears overall somewhat less aggressive than other types of angiosarcoma, it carries a high risk of local recurrence, metastasis and death. In this series, there is no correlation between histologic tumor grade and patient outcome, more in line with angiosarcomas at other sites.

176 Loss of Myoepithelium and Laminin at the Tumor-Stromal Interface in Circumscribed Solid Papillary Carcinoma of the Breast

MM Nicolas, Y Wu, LP Middleton, MZ Gilcrease. M. D. Anderson Cancer Center, Houston, TX.
Background: Solid papillary carcinoma (SPC) is an unusual variant of papillary carcinoma with a predominantly solid pattern of expansile intraductal growth with interspersed fibrovascular cores. Circumscribed lesions are generally considered to be noninvasive, but previous studies have reported contradictory data on the frequency of myoepithelial loss at the periphery of these expansile lesions. In an attempt to clarify the frequency of myoepithelial loss in circumscribed solid papillary carcinomas of the breast, we performed immunohistochemical staining for myoepithelial markers on a series of SPC cases with circumscribed foci that appeared to be noninvasive by H&E morphology. Because integrity of the basement membrane has also been used as a criterion to support lack of stromal invasion, staining for basement membrane protein was also performed on each case.
Design: Eleven cases of SPC with available blocks or unstained slides were retrieved from the M. D. Anderson archives or obtained from outside contributors. Seven of these were diagnosed as entirely noninvasive or as only microinvasive. The remaining 4 had larger amounts of associated overt invasive tumor. Immunohistochemical staining for smooth muscle actin (SMA), p63, and laminin was performed on each case, and staining was evaluated on the circumscribed nests that appeared to be noninvasive by H&E morphology.
Results: Seven of the 11 cases (64%) were entirely negative for both SMA and p63 at the periphery of at least 1 circumscribed nest that appeared noninvasive by H&E morphology. Five of these had at least one circumscribed focus that lacked both myoepithelial markers and laminin staining along the entire tumor-stromal interface. One of these 5 cases was diagnosed with only microvasion, but 2 of the ipsilateral lymph nodes were found to contain metastatic tumor that resembled the circumscribed primary tumor. Lymph nodes from the remaining cases with axillary node sampling (6 cases) were either negative or contained only isolated keratin-positive tumor cells.
Conclusions: Circumscribed SPC of the breast not infrequently lacks both myoepithelial markers and laminin at the tumor-stromal interface. Because metastases have been reported from such tumors, they might be invasive in spite of a circumscribed noninvasive appearance. Immunostains for both myoepithelial markers and laminin might be helpful in identifying circumscribed tumors with metastatic potential.

177 Comparative Study of the Profile of Primary DCIS and Subsequent In Situ or Invasive Recurrence

S Nofech-Mozes, J Spayne, E Rakovitch, L Lickley, HJ Kahn, W Hanna. Sunnybrook Health Sciences Centre, Toronto, ON, Canada.
Background: Data regarding the morphology and the expression of ER, PR, HER2/neu oncoprotein in primary ductal carcinoma in situ (DCIS) and subsequent recurrent tumor in the same patients is limited.
Design: We studied a cohort of 133 cases with pure DCIS treated with breast conserving surgery alone, between 1982-2000. Ipsilateral recurrence occurred in 41 cases. We reviewed the histology and assessed the expression of ER , PR and HER2/neu by immunohistochemistry.
Results: Median age at diagnosis was 52. The median follow up was 11 yrs. *Cases with DCIS recurrence* (n=20; blocks available 17; median time to recurrence- 2.7 yrs): The nuclear grade was concordant in 90% (18/20), and comedo necrosis in 85% (17/20). The expression profile of ER/PR HER2/neu was identical in 58.8% (10/17). ER status changed in 2 cases (1 gain and 1 loss). PR status changed in 4 cases (1 gain and 3 losses). Two cases showed loss of HER2/neu overexpression and a third case showed a gain of HER2/neu overexpression coupled by loss of PR, and higher nuclear grade. *Cases with invasive recurrence* (n=21; blocks available 19; median time to recurrence- 5.6 yrs): nuclear grade was concordant in 57%(12/21). 7/9 discordant cases showed higher nuclear grade in the recurrence. The expression profile of ER/PR HER2/neu was identical in 57.8%(11/19). ER status changed in 4 cases (3 gains and one loss). One case showed loss of PR. HER2/neu overexpression was lost in 4 cases and gained in one. In one case loss of HER2/neu overexpression was coupled by gain of PR. A triple negative DCIS recurred as triple-negative invasive carcinoma.
Conclusions: A high level of concordance in nuclear grade and comedo necrosis is seen in tumors that recurred as DCIS whereas there was a significant difference in nuclear grade in cases with invasive recurrence (p=0.018). In both types of recurrences, the discordant cases showed a tendency for recurrent tumors to be of a higher nuclear grade than the primaries. A high level of concordance was maintained when each marker was

assessed individually, however; the concordance of the panel of biological markers was noted only in about 60% of the cases. Gain or loss of expression of molecular markers occurred not only in tumors recurring as invasive carcinoma but also in cases that maintain their in situ histology. These findings in this unique cohort are without the impact of radiation or systemic therapy . Our findings suggest that the biological profile should be re-evaluated on recurrent tumors.

178 Breast Cancer after Irradiation for Hodgkin's Lymphoma

L Orta, I Bleiweiss, C Nagi, S Jaffer. Mount Sinai School of Medicine, New York, NY.

Background: Advances in treating Hodgkin's lymphoma (HL) have led to improved survival. Exposure to previously used mantle radiation (RT) increases the risk of breast carcinoma in younger patients. Since these patients now undergo more frequent breast imaging surveillance, we studied the characteristics of HL/RT-related breast carcinoma presenting within the last decade.

Design: We searched our computerized pathology records for patients diagnosed with a breast carcinoma during the years 1994 to 2006 after receiving HL/RT.

Results: Twenty patients were identified. The median age of diagnosis of breast cancer was 43 years (range 30-64). Thirteen patients (65%) were irradiated before the age of 35. The median latency period was 20.5 years (range 12-30). 9 patients had mastectomy, 6 with prophylactic contralateral mastectomies. Unsuspected disease was present in 2 prophylactic mastectomies, one with 2 separate invasive poorly differentiated duct carcinomas (1.2cm and 0.6cm) and one with intraductal carcinoma (DCIS). 6 patients were treated with wide excision, and 4 had core needle biopsies only. 17 invasive carcinomas were found: 12 ductal (5 moderately differentiated and 7 poorly), 2 lobular (1 each of alveolar and pleomorphic types) and one each of mixed poorly differentiated duct and lobular, squamous cell, and colloid. The invasive tumors ranged from 0.1cm to 4.8cm (median 0.8). Two patients (10%) developed synchronous ipsilateral duct carcinomas. Bilateral disease was present in 3 (15%). Five patients presented with DCIS only. Two out of nine invasive carcinomas were positive for Her-2neu, 9/11 positive for ER and 8/11 positive for PR. Axillary dissection was done in 8 cases, with none showing metastases; however, the patient with unsuspected carcinoma in the prophylactic mastectomy developed axillary metastases with extranodal extension 6 months after mastectomy and subsequent axillary recurrence. Sentinel lymph nodes were obtained in five cases; none showed metastases. One patient had occult breast cancer after axillary node presentation. Stage at presentation was as follows: Stage 0 5/20 (25%), Stage I 13/20 (65%), Stage IIA 2/20 (10%).

Conclusions: Most studies of breast carcinoma arising after HL/RT were published before recommendations for earlier and more frequent mammographic surveillance were in place, and contained larger tumors presenting at later stage with more frequent bilateral disease. Our results indicate that early surveillance protocols and new imaging modalities might be responsible for the earlier stage at presentation in these women.

179 Anti HER2 Rabbit Monoclonal Antibody SP3 Is a Better Predictor of HER2 Gene Status in Invasive Breast Carcinoma

E Orvieto, L Zanatta, S Rossi, A Furlanetto, F Canal, L Laurino, AP Dei Tos. General Hospital, Treviso, Italy.

Background: The assessment of HER2 status in breast cancer plays a major role in selecting patients for anti HER2 therapy. Correlation between immunohistochemistry and FISH is considered optimal for 0, 1+ and 3+ cases. By contrast 2+ cases represent a "gray" area in which gene status assessment is usually considered mandatory. The establishment of effective diagnostic algorithms as well as of reproducible scoring systems remain source of sharp debate.

Design: One hundred 2+ HER2 expressing invasive ductal breast carcinomas (as assessed by HercepTest, Dako, Glostrup, Denmark)) were retrieved from the files of the Department of Pathology of Treviso, Italy, and immunostained with a commercially available anti HER2 rabbit monoclonal antibody (RabMAb) (clone SP3, LabVision, Fremont, CA). All cases were scored using the FDA endorsed scoring system included in the HercepTest. The HER2 gene status was analyzed in all cases by FISH (PathVision, Vysis, Downers Grove, IL).

Results: FISH analysis showed HER2 gene amplification in 9/100 (9%) of cases. Using the SP3 RabMAb 90 out of 100 HercepTest 2+ cases were scored 0/1+, 8 out of 100 2+, and 2 out of 100 3+. The correlation between SP3 expression and HER2 gene amplification is shown in Table 1. The single SP3 negative case actually showed less than 10% HER2 expressing neoplastic cells and was therefore scored as "negative" according to the FDA approved scoring system.

Conclusions: 1. Anti HER2 RabMAb SP3 exhibit much greater accuracy in predicting HER2 gene status. **2.** A dramatic reduction of HER2+ positive cases is observed with consequent reduction of redundant FISH analyses. **3.** The detection of rare cases showing less than 10% HER2 overexpressing/amplified neoplastic cells should prompt a reappraisal of currently available scoring systems.

Table 1

HER2 Score	SP3 RabMAb	HER2 amplification
0-1+ "negative"	90/100	1/90
2+	8/100	6/8
3+	2/100	2/2

180 Automated Accelerated Silver In-Situ Hybridization (SISH™) for Detection of *HER2* Gene Amplification in Breast Carcinoma

BG Papouchado, E Downs-Kelly, L Pestic-Dragovich, S Hood, JD Pettay, J Myles, M Loftus, N Prescott, T Grogan, PC Roche, AS McElhinny, DJ Dabbs, WM Hanna, M Dietel, DG Hicks, R Alsabeh, WC Powell, RR Tubbs. Cleveland Clinic, Cleveland, OH; Tucson, AZ; Pittsburgh, PA; Toronto, CA; Charite & Universitatsmedizin, Berlin, Germany; Roswell Park Cancer Institute, Buffalo, NY; Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Determination of *HER2* status is crucial for determining management of breast carcinoma. Use of FISH as the primary method for determining *HER2* status may be problematic, requiring specialized instrumentation and training. Bright field in situ hybridization is readily incorporated into surgical pathology workflow, but utility has been limited by manual methods requiring overnight hybridization.

Design: 96 invasive breast carcinomas for which FISH results were known were analyzed for *HER2* and chromosome 17 (CP17) status by SISH™. Challenging cases (monosomic and polysomic CP17, *HER*2 monoallelic deletion, genotypic heterogeneity) comprised ~20% of the evaluated cases. SISH and FISH signal enumeration was performed; *HER2* gene amplification was defined as *HER2*/CP17 ratio ≥2. SISH slides also were semiquantitatively scored as *HER2* amplified, heterogeneous, or nonamplified by nine pathologists. *HER2* FISH status was used as the reference standard (multiple regression analysis for spot counts, kappa for observer agreement).

Results: There was nearly perfect agreement (97.9%) between *HER2* SISH and the corresponding FISH counts ($R^2 = 0.955$, $p>0.001$; sensitivity 92%/specificity 100%). Interpretation of the SISH results compared with FISH were highly reproducible among the nine pathologists [average kappa 0.812 (amplified versus non amplified cases) and 0.777 (amplified and heterogeneous cases versus non amplified cases)].

Conclusions: SISH *HER2* results are highly concordant with FISH, and interpretation is reproducible. SISH staining uses a fully automated protocol, a repeat free *HER*2 probe, a CP17 centromeric reference, and accelerated detection providing a *HER*2 genotype in approximately six hours. Standard bright field microscopic SISH interpretation is readily integrated into surgical pathology workflow for routine assessment of *HER2* status in breast carcinoma.

181 Keratin Immunohistochemistry Does Not Contribute To Correct Lymph Node Staging in Patients with Invasive Lobular Carcinoma

DT Patil, CL Schiller, B Susnik. Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: Infiltrating lobular carcinoma (ILC) is notorious for single cell intraparenchymal nodal metastases. Although according to the present recommendations of the College of American Pathologists immunohistochemical staining (IHC) of sentinel lymph nodes (SLN) with keratin is not mandatory, it has been suggested that IHC improves rates of detection of nodal metastases and is needed for adequate staging in lobular carcinoma. The aim of the present study was to evaluate the value of cytokeratin IHC in detecting SLN metastases and its effect on lymph node staging in patients with ILC.

Design: Histological material from 77 consecutive cases of ILC undergoing SLN biopsy procedure at a single institution were retrieved. Hematoxilyn and eosin (H&E) stained slides of primary tumor and SLN (three levels per block) were reviewed. IHC (AE1/AE3) was performed on negative nodes and metastatic deposits were classified as macrometastasis (> 2.0 mm), micrometastasis (>0.2 – 2 mm) and ITC (isolated tumor cells, ≤ 0.2 mm). Lymph nodes with ITC were interpreted as negative for metastasis (AJCC staging manual, 6th edition). The relationship between lymph node status and tumor size was also analyzed.

Results: A total of 195 SLN from 77 patients were evaluated. The median age was 59 years (range: 38- 83 years). Of the 77 cases, 25 (32.5%) were positive for metastases and 14 (18.2%) for ITC. The metastatic deposits included 21 macro and 4 micrometastases. H&E alone identified all macrometastases (21) and 3 of 4 micrometastases. All ITC and 1 micrometastasis were detected by IHC only. Based on IHC results, the lymph node stage was altered in 1/77 cases (1.3%). The micrometastasis in this case was associated with a T3 tumor. The proportion of lymph nodes involved by micro or macrometastases increased with tumor size. Increasing tumor size was also associated with a higher proportion of lymph nodes exhibiting ITC.

Conclusions: In our group of 77 patients, cytokeratin IHC resulted in change of nodal status and stage in a single patient (1.3%). These results indicate that cytokeratin IHC does not provide any significant advantage over conventional histological examination of sentinel lymph nodes in lobular carcinoma and its routine use is not warranted. However, it may be worth considering this additional step in patients with large primary tumors.

182 Subtypes of Invasive Breast Carcinoma and Pathologic Response to Neoadjuvant Chemotherapy

G Peiró, FI Aranda, E Adrover, M Planelles, C Alenda, A Payá, J Seguí. Hospital General Universitari, Alacant, Spain.

Background: Breast carcinoma (BC) subtypes classified according to their gene expression differ in biology and behaviour. However, the association with response to neoadjuvant chemotherapy (NACT) is currently not well known.

Design: We studied 150 core needle biopsies and the corresponding resection specimens of BC and axillary lymph nodes. Patients received NACT with Anthracycline +/- Taxanes-containing protocols. Pathologic response (pR) was assessed in H&E as the percentage of invasive cells and classified as "non response" (no changes or tumor reduction ≤90%) and "response" (reduction >90% +/- in situ carcinoma). Immunohistochemistry for hormone receptors (HR) (estrogen and progesterone), HER2, CK5/6, p53 and Ki67 were performed. HER2 gene status was confirmed by FISH (DakoCytomation pharmaDx) in 2(+) cases.

Results: The mean age was 48 years (range 24-77). Of the 150 BC, 13 (8.7%) were lobular and 87 (91.3%) ductal: 51 (34%) exhibited the luminal subtype A (HR>50% and/or Ki67>30%), 20 (13.3%) subtype B (HR<50% and/or Ki67≥30%), 41 (27.3%) HER2+ and 25 (16.7%) basal-like (HER2-/HR-) phenotype, which included 19 (76%) with CK5/6+. p53 overexpression in >50% cells was present in 48% basal-like and in 39% HER2+ tumors (p=0.0001). Pathologic response (pR) was seen in 39% cases, that was complete in 19.3%, associated with grade 3 (p=0.027) and the presence of necrosis (p=0.041). Among subtypes, HER2+ showed significantly higher pR (61%) followed by basal-like (44%), luminal B (40%), luminal A (25%) and lobular carcinoma (7.7%) (p=0.001). 44% patients had pathologically negative axillary lymph nodes, including 9% with fibrous stromal changes, which were more frequently seen in HER2+ tumors (p=0.008).
Conclusions: Subclassification of BC is of interest since they show distinct differences in response to therapy, with HER2+ subtype showing the highest rate. *Supported by grant FIS 03/1411*.

183 Topoisomerase II-alpha Gene Status and Pathologic Response to Neoadjuvant Chemotherapy in HER2-Positive Breast Carcinoma

G Peiró, FI Aranda, E Adrover, FM Peiró, A Abuomar, M Niveiro, S Benlloch. Hospital General Universitari, Alacant, Spain.
Background: HER2 oncogene status has been correlated with a better response in patients with breast carcinoma (BC) treated with anthracyclines-based adjuvant chemotherapy. However, its predictive value of pathologic response (pR) in patients with neoadjuvant chemotherapy (NACT) in association with Topoisomerase II-alpha (TOP2A) gene alterations is not well known.
Design: Of 150 patients with BC in NACT with anthracycline +/- taxanes-containing protocols, we selected 42 cases with HER2-positive (+) status. We recorded the pathologic data from core needle biopsies and resection specimens of BC and axillary lymph nodes. pR assessed in H&E as the percentage of invasive cells, was classified as "non response" (no tumor changes or reduction ≤90%) and "response" (reduction >90% +/- in situ carcinoma). HER2 (Novocastra), p53 and Ki67 (DakoCytomation) protein expression were studied by immunohistochemistry (IHC) and HER2 gene status was confirmed by FISH (DakoCytomation pharmaDx) in 2(+) cases. TOP2A gene amplification was determined by chromogenic in situ hybridization (CISH; Zymed). The relationship between TOP2A, pathologic and IHC data was analyzed.
Results: HER2+ tumors were more frequently of grade 3 (76%), showing necrosis (69%), LVI (24%), Ki67 ≥30% (50%), p53 ≥50% (38%) and patients were younger than 50 years (62%). The tumor response rate was 59.5% (25/42) and in 31% (13/42) was complete (absence of invasive carcinoma). Axillary lymph nodes were negative in 52% cases, including 21% with fibrous stromal changes. TOP2A amplification (≥6 copies in ≥30% of nuclei) was seen in 14 (33.3%) tumors. No differences were found between TOP2A status and age, grade, presence of necrosis, LVI, lymph node involvement, Ki67 or p53 expression (all p>0.05). We observed a trend towards an increased pR in patients whose tumors had no amplification of TOP2A (60%; 15/25) compared with those with amplification (40%; 10/25) (p=0.26).
Conclusions: In patients with HER2+ BC treated with anthracyclines-based NACT, the subset of tumors that show amplification of TOP2A appears to have more extensive residual disease. Therefore, they may benefit from alternative treatment strategies. *Supported by grant FIS 03/1411*.

184 The FISH 2002 Study: Comparison of Techniques To Assess the HER2 Status of Patients with Metastatic Breast Cancer

F Penault-Llorca, L Arnould, G MacGrogan, A Leroux, I Treilleux, M Antoine, MO Vilain, C Blanc-Fournier, F Ettore, V Fermeaux, C Charpin-Taranger, J Jacquemier, B Lannes, G Escourrou, M Lacroix-Triki, P Gosset, C Sagan, J Couturier, A Vincent-Salomon. GEFPICS, Cedex, France.
Background: Immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH) can be used to assess human epidermal growth factor receptor 2 (HER2) status and identify patients with breast cancer who are eligible for trastuzumab (Herceptin®) treatment. The FISH 2002 study investigated concordance between IHC and FISH performed in peripheral and regional reference centres.
Design: HER2 IHC was performed on primary breast tumours of patients presenting with metastatic breast cancer. Paraffin-embedded tumour samples and initial IHC results from peripheral centres were sent to their corresponding regional reference centres where IHC was repeated and FISH performed to determine concordance with IHC. Assessments of concordance excluded samples with equivocal IHC scores (2+ according to the HercepTest® scoring system).
Results: From December 2002 until June 2006, 18 regional reference and 73 peripheral centres collected data from 1543 patients. We present here the interim analysis of the first 1153 patients. In regional reference centres, the HER2 overexpression rate (IHC 3+) was 23.6% (177/751) and 15.2% (114/751) of samples were IHC 2+. The IHC false-positive rate (IHC 3+, not amplified) was 2.3% and the false-negative rate (IHC 0 or 1+, amplified) was 1.5%. In peripheral centres, the HER2 overexpression rate was 19.2% (113/587) and 20.4% (120/587) of samples were IHC 2+. The IHC false-positive and false-negative rates were 13.5% and 3.7%, respectively. FISH analysis identified 27.4% (363/1326) of samples as FISH positive. Considering all IHC 0/1+ and 3+ cases, the rate of discordance between IHC and FISH was 2.3% (n=1153). The advantages and disadvantages of each technique will be presented at the meeting.
Conclusions: Concordance between IHC and FISH was high despite differences between centre protocols. While the rate of false negatives for IHC was low, the higher rate of false positives emphasises the need for greater accuracy of IHC testing. Quality-assurance programmes are essential in achieving high-quality HER2 testing to ensure optimal treatment for each patient.

185 Invasive Breast Carcinoma in Young Patients: Phenotypes and Correlation with Cell Cycle Proteins and Survival

M Planelles, FI Aranda, M Niveiro, G Peiro, A Abuomar. Hospital General de Alicante, Alicante, Spain.
Background: Microarray profiling of invasive breast carcinomas (BC) has identified distinct subtypes of tumors (Luminal A, Luminal B, HER2 overexpressing and basal like), which are associated with different clinical outcomes. The aim of our study was to determine the frequencies of these phenotypes in a series of BC in patients 40 years old or younger, and their correlation with the expression of several proteins involved in the cell cycle progression, such as Cyclin D1, p21 and p27.
Design: Formalin-fixed paraffin-embedded tissue from 90 BC in women ≤ 40 years were retrieved from the archives of the Department of Pathology at the General Hospital of Alicante (Spain). Two 1mm cores were taken from separate areas of each tumor. Two tissue microarray blocks were created. Immunohistochemistry for hormone receptors (ER/PR), HER2, Ki67, HER1, CK5/6, Cyclin D1, p21 and p27 were performed. Tumors were classified as (a) luminal A (RE/RP+; HER2-; Ki-67<20%), (b) Luminal B (RE/RP+; HER2-; Ki67>20%); (c) HER2+; (d) basal-like (CK5/6 and/or HER1+), and (e) indeterminate (HER2/RE/RP/HER1/CK5/6-). Significant associations were identified using Chi-square and Fisher´s exact test. Survival was calculated by the Kaplan-Meier method (log rank test). P value ≤0.05 was considered significant.
Results: Mean age of the patients was 34 years (range 20-40 years). The distribution of phenotypes was as follows: 37 (41%) tumors were Luminal A, 17 (19%) Luminal B, 8 (9%) HER2+, 22 (24%) basal-like and 6 (7%) were indeterminate. Among them, the Luminal A and B showed higher expression of p21 (≥1% positive nuclei) (39.5%, and 31.6%, respectively; p<0.043) and Cyclin D1 (≥10% positive nuclei) (51.2% and 29.3%, respectively; p=0.000), but no significant association was found with p27 (p=ns). Survival analysis showed a trend towards a poorer survival for those patients with tumors with Cyclin D1 overexpression (p= 0.06). However, there was no difference regarding tumor phenotypes or the levels of p21 and p27 (p=ns).
Conclusions: In our series of breast carcinoma in young patients (≤40 years), Luminal A phenotype was the most frequent, but there was no correlation with survival. However, increased expression of Cyclin D1 appeared to correlate with poor outcome.

186 Genomic Profiling of Heterologous Metaplastic Breast Carcinoma (MBC) Show Clonal Similarities between Different Lesion Types but Also Distinct Differences Which Might Account for Their Morphological Features

DG Powe, M Ademola, MBK Lambros, T Abdel-Fatah, IO Ellis, JS Reis-Filoh. Nottingham University, Nottingham, United Kingdom; Institute of Cancer Research, London, United Kingdom.
Background: Metaplastic breast carcinoma (MBC) is a complex disease comprising a heterogeneous mix of morphologically distinct epithelial and mesenchymal-type lesions. Fundamental questions remain concerning whether the different lesion types derive from the same progenitor cell, and if it ectodermal-derived lesions can give rise to a malignant mesenchymal cell lineage. In this study, we sought to address these questions by comparing the genomic profiles between morphologically distinct regions in a single case of high grade MBC.
Design: Genomic expression profiles of the four morphologically distinct regions were determined using array comparative genomic hybridization (aCGH) on amplified laser microdissected DNA. Separate samples of DCIS-like, spindle cell, epithelioid/rhabdoid, and chondroid DNA were fluorescently labelled, combined with normal female reference DNA, and used as probes on a whole genome high-resolution 32K BAC array. The balance in fluorescence signal intensity between each pair of tumour (test) and normal (reference) DNAs along each chromosome was analysed using cluster analysis and by comparing karyotypes for each lesion.
Results: All four lesions shared chromosomal gains at chr1q, 2q, 3q, 7q, 8q & 13q. Common losses were seen at chr1p, 5q, 9p, 10q, 11q & 17p. A number of putative tumour suppressor genes (TSG) including those with cell adhesion, pro-apoptotic and mitotic spindle assembly functions were identified corresponding to the deleted regions. Of the amplified regions, genes involved in calcium mediated cell transduction, cell proliferation and stromal remodelling were found. In addition, we identified a further 9-12 different chromosomal alterations confined to each lesion type.
Conclusions: All four MBC morphological regions shared major genomic imbalances supporting the concept that they have a common clonal origin and that these alterations may be important in the development of metaplastic breast carcinoma. However, distinct region-specific / morphology-specific identities were apparent which also supports the concept of a direct relationship been morphological structure and underlying molecular genetics. The mechanism for development of genetic and morphological heterogeneity with metaplastic breast carcinoma merits further investigation.

187 A New Rabbit Monoclonal Antibody (RMab), 4B5, for the IHC Determination of *HER2* Status in Breast Cancer: Comparison with CB11 and FISH with Interlaboratory Reproducibility (IR)

WC Powell, DG Hicks, N Prescott, J Pettay, RB Nagle, DJ Dabbs, KM Scott, RW Brown, T Grogan, PC Roche, R Tubbs. Ventana Medical Systems, Inc, Tucson, AZ; Roswell Park Cancer Institute, Buffalo, NY; Cleveland Clinic Foundation, Cleveland, OH; University of Arizona Health Sciences Center, Tucson, AZ; UPMC-Magee Women's Hospital, Pittsburgh, PA; University of Texas Health Science Center Medical School, Houston, TX.
Background: The two methodologies in clinical use to assess *HER2* status in breast cancer are: FISH (gene amp) and IHC (protein over-expression). A consistent finding has been that 3-15% of breast cancers over-express *HER2* protein without evidence for gene amplification. Accurate determination of the *HER2* status has implications for selecting patients most likely to respond to Trastuzumab. We report here our experience with a new anti-*HER2* RMab, 4B5.

Design: The evaluation of *HER2* status in two different cohorts of breast cancer cases (Single institution (SI) and multinational (MN)) with a total of 322 breast cancer cases was performed on an automated staining system (Ventana Medical Systems) and scored by 3 pathologists (0-3+), for comparison with CB11 staining results, and FISH. IR of staining and interpretation was determined on a subset of the SI based cohort at 3 laboratories.
Results: RMab 4B5 demonstrated sharper membrane staining with less cytoplasmic and stromal background staining than CB11. In the SI cohort, the staining results for 4B5 were highly comparable to those obtained for CB11 with an overall concordance of 93.3%. In the MN cohort, the concordance with CB11 was 84.7%. However, 4B5 had a much higher overall agreement with FISH (88.2%), compared to agreement of CB11 with FISH (81.2%). The difference in the performance of CB11 in the MN cohort versus the SI cohort may be due to differences in tissue fixation and processing in a centralized, high volume laboratory in an academic medical center versus multiple sites in the international community with potentially non-standardized techniques.
Conclusions: The results with 4B5 indicate that it has a more robust performance than CB11 since the correlation of 4B5 with FISH was nearly equivalent (88.2% MN; 89.3% SI) in both cohorts. IR was also excellent (Kappa 1.0). RMab 4B5 provides excellent sensitivity, specificity, and IR for the detection of *HER2* status in breast cancer.

188 Columnar Cell Lesions (CCL) with and without Atypia in Needle Core Biopsy of the Breast: When Is Excision Appropriate?

KE Purdy, A Nassar, S Logani. Emory University School of Medicine, Atlanta, GA.
Background: The terminology and criteria used to define CCL with atypia have been only recently refined. There is limited data on follow up excision for a diagnosis of flat epithelial atypia (FEA) on needle core biopsy (NCB). The objectives of the current study were to review NCB diagnosed as CCL with and without atypia using currently accepted criteria and to correlate with findings on excision biopsy (EB).
Design: 219 NCB coded as CCL were retrieved from the Emory pathology files from 2002 to 2006. Only patients with follow up EB were included (n=63). To obtain a pure case set in which excision was performed solely for a diagnosis of FEA or CCL with architectural features of ADH, we excluded all NCB with ductal or lobular carcinoma in situ (DCIS, LCIS) or invasive carcinoma (n=19), intraductal papilloma (n=4), atypical lobular hyperplasia (n=5) and cases with discordant biopsy and mammographic findings (n=8). 18 patients had excision for FEA and 9 underwent excision for CCL with architectural features of ADH, providing a total of 27 patients. All slides were reviewed by two pathologists using currently proposed criteria and the results correlated with findings on EB. A minimum threshold of DCIS or LCIS was established as a significant lesion on EB.
Results: The original pathology reports revealed a wide range of terminology for FEA. On review, 6 NCB previously diagnosed as FEA were downgraded to CCL without atypia, and 1 was diagnosed as CCL with ADH. One case initially reported as CCL with ADH was judged to contain FEA. This provided a study group of 12 FEA, 8 CCL without atypia, and 7 CCL with ADH. 2 of 12 (16.6%) with FEA on NCB showed a clinically significant lesion on EB: 1 low grade DCIS and 1 case of grade 1 tubulo-lobular invasive carcinoma. In contrast, none of the patients with a review diagnosis of CCL alone showed a significant lesion on EB. 1 of 7 (14%) of the cases with architectural atypia in the background of CCL demonstrated low grade DCIS on EB.
Conclusions: The practical application of diagnostic criteria for FEA on NCB showed considerable interobservor variability, further compounded by the lack of standardized reporting terminology. The presence of cytologic atypia (FEA) or architectural abnormality (ADH) in the background of CCL confers an increased risk of a biologically significant lesion on EB. Patients with FEA or ADH in the background of CCL should be advised to undergo excision to exclude the possibility of a malignancy, which is more likely to be low grade in situ or invasive carcinoma.

189 Discordant Gross and Microscopic Pathology Following Neoadjuvant Treatment of Breast Cancer: Implications for Evaluating Tumor Size and Margin Status

JT Rabban, YY Chen. University of California, San Francisco, San Francisco, CA.
Background: Neoadjuvant chemotherapy (NACT) for advanced breast cancer may alter the microscopic appearance of residual tumor cells (low cellularity, apocrine cytoplasmic changes) and background stroma. Correlation between microscopic and gross specimen findings is not well studied but may affect pathologic examination of the NACT treated breast. We evaluated this correlation and its implications for determining residual tumor size and margin status.
Design: Slides from 127 patients with NACT treated invasive breast cancer were retrospectively reviewed. Gross specimen findings were classified as unremarkable, altered stroma (vaguely distributed stromal fibrosis but no mass), or residual mass. Microscopic findings were classified as residual invasive carcinoma (InvC), ductal carcinoma in-situ (DCIS) or benign. InvC was classified as unifocal or multifocal. NACT treatment change was defined as sparse cellularity, apocrine cytoplasmic change, or tight small clusters of a few tumor cells with minimal cytoplasm.
Results: Gross findings were unremarkable in 23/127. Of these, 12 (52%) had InvC (avg size 3.5 cm, max 12 cm, 50% multifocal) and 2 had DCIS only. Ten (83%) InvC showed NACT treatment changes. Margins were positive in 4 (33%). Grossly altered stroma without a mass was seen in 27/127 cases. Of these, 15 (56%) had InvC (avg size 3.3 cm, max 11.1 cm., 53% multifocal) and 5 had DCIS only. Nine (60%) InvC had NACT treatment changes. Margins were positive in 2 (13%). Gross residual mass was seen in 77/127 cases. Of these, 76 (99%) had InvC (avg size 4.4 cm, max 12.4 cm) and 1 had DCIS only. Margins were positive in 18 (24%).
Conclusions: Significant residual invasive carcinoma can be present in specimens following NACT despite a complete absence of gross evidence of disease. Most of such occult carcinoma shows notable treatment changes microscopically and half are multifocal, possibly explaining the high rate of positive surgical margins. Careful tissue sampling over the initial pre-treatment dimension of tumor is required even in the absence of gross pathology. Attention to sampling margins is also warranted given the risk of occult positive margins.

190 Non-Sentinel Node Status in Breast Cancer Patients with Isolated Tumor Cells in the Sentinel Node: Implications for Axillary Node Dissection

PB Rajan, E Rajan, K Yao. Loyola University Medical Center, Maywood, IL; Loyola University Cardinal Bernardin Cancer Center, Maywood, IL.
Background: Isolated tumor cells (ITC) are defined as single tumor cells or small clusters of cells that are not greater than 0.2 mm in size and that usually show lack of malignant activity (AJCC Staging System, 2002). Controversy exists regarding axillary dissection (ALND) for sentinel node (SLN) metastases detected as isolated tumor cells (ITC). We hypothesize that the number of positive non-SLN is absent or low and ALND is unnecessary for patients with ITC.
Design: This study was based on 30 breast cancer patients between July 1999 and August 2006 with sentinel node ITC detected either on routine hematoxylin and eosin (H&E) and/or on immunohistochemical stains (IHC). ALND was performed in 8 of 30 patients. The age at diagnosis, type of tumor, primary tumor size (extent in case of ductal carcinoma in situ, DCIS), size of ITC, number of examined SLNs and non-SLNs, and follow-up status were recorded.
Results: The age of patients ranged from 41 to 82 years. There were 27 cases of infiltrating carcinomas, one case of DCIS with microinvasion and 2 cases of pure DCIS. All 3 DCIS revealed high grade comedo type morphology with tumor extent ranging from 2.3 to 5.0 cm. The size of invasive tumors ranged from 0.2 cm to 2.5 cm and the size of ITC in SLN was between 0.1 mm to 0.2 mm. The number of SLNs examined varied from 1 to 7. Of the 30 patients, ALND was performed in 8 invasive carcinoma patients. The number of non-SLN ranged from 7 to 39. There was no evidence of non-SLN disease in 7 out of 8 patients with ALND. In one patient with ALND there was a focus of ITC in one out of 12 non-SLNs examined. There was no evidence of subsequent axillary and/or systemic recurrences in any of the 30 patients and all the patients were alive and well on last follow-up.
Conclusions: Axillary lymph node dissection may not be necessary in breast cancer patients when the SLN is positive for ITC. However, the findings of large trials such as NSABP B-32 are necessary to confirm this observation and to determine the optimal management of these patients.

191 Evaluation of Hormone Receptor Status in Breast Cancer Using New Progesterone Receptor Antibody

AB Rajput, MC Cheang, DK Voduc, DA Turbin, S Leung, KA Gelmon, AM Gown, BC Gilks, DG Huntsman. Genetic Pathology Evaluation Centre, Vancouver, BC, Canada; University of British Columbia, Vancouver, BC, Canada; Phenopath Laboratories, Seattle, WA.
Background: There is controversy regarding the utility of progesterone receptor (PR) staining in immunohistochemical (IHC) evaluation of hormone receptor positive breast cancer. We wanted to determine whether assessment of PR status using a new rabbit anti-PR monoclonal antibody (mAB) provides important additional information, when estrogen receptor (ER) status is known.
Design: Clinically annotated tissue microarrays (TMAs) were constructed from 1812 breast cancer patients. IHC assessment of PR status was done using the newly developed rabbit mAB anti-PR (1E2) on the Ventana automated slide stainer. The fractions of PR positive tumor nuclei were scored as 0 (<1%), 1 (1-25%), 2 (25-75%), and 3 (>75%). Breast cancer specific survival was analyzed by KM method and log rank test was used to compare survival curves.
Results: 51.6% of cases were positive for PR with a mean survival time of 14.3 years. PR status was a powerful favorable ($p=7.03\times10^{-11}$) prognostic factor. There were 857 (47.3%) ER+/PR+ tumors, 413 (22.8%) ER+/PR- tumors, 78 (4.3%) ER-/PR+ tumors, and 464 (25.6%) ER-/PR- tumors. The ER+/PR- and ER-/PR+ cases had an intermediate prognosis, significantly worse than the ER+/PR+ cases ($p<0.001$ and $p=0.035$, respectively). The ER+/PR- cases had a significantly better prognosis than the ER-/PR- cases ($p=0.019$) but the ER-/PR+ tumors appear to have an intermediate survival compared to the ER-/PR- cases, which did not reach statistical significance ($p=0.21$). In a subset of 574 patients treated with tamoxifen only, the patients with ER+/PR- tumors had a significantly worse prognosis than the ER+/PR+ cases ($p=0.035$). There were only 11 ER-/PR+ tumors in this group, precluding assessment of prognostic significance of ER-/PR+ status in patients treated with tamoxifen. Considering all cases, the expression of ER was significantly lower in the ER+/PR- group, compared to the ER+/PR+ group.
Conclusions: PR+ status is associated with a better prognosis, and can be used to stratify ER+ tumors into groups with significantly different outcomes. ER-/PR+ tumors are uncommon and are associated with a prognosis similar to that of ER+/PR- cases.

192 A Mouse Model of Basal-Like Breast Carcinoma with Metaplastic Elements

JS Reis-Filho, A McCarthy, K Savage, A Gabriel, C Naceur, A Ashworth. Institute of Cancer Research, London, United Kingdom.
Background: Tumours arising in patients with *BRCA1* germline mutations frequently have a basal-like phenotype, being characterised by high histological grade, central necrotic areas, foci with metaplastic differentiation, lack of hormone receptors and HER2 expression, and consistent positivity for basal markers, including cytokeratin (CK) 5/6, CK14 and epidermal growth factor receptor (EGFR). Although basal-like breast carcinomas have received great attention in the literature, no targets for tailored therapy have so far been identified for this group of tumours. Furthermore, no animal models

for the study of these tumours are available. The aims of this study were to describe an animal model for basal-like breast carcinomas and to compare the histological and immunohistochemical features of these tumours with those reported for human basal-like breast carcinomas.

Design: We have used Cre-loxP technology to generate a mouse model of *Brca1*, targeting the last BRCT domain of *Brca1*, which upon recombination would produce a truncated protein similar to that seen in human disease. The specific loss of Brca1 in the mammary gland leads to tumour formation on both a wildtype and heterozygous p53 background. Twelve tumours from 8 animals were subjected to thorough morphological analysis and immunohistochemistry with antibodies against oestrogen receptor, HER2, epidermal growth factor receptor. The features of these tumours were compared with those reported for human sporadic basal-like carcinomas and tumours arising in BRCA1 mutation carriers.

Results: On histological analysis, these mammary gland tumours were characterised by high histological grade, central necrotic areas and presence of homologous metaplastic elements, morphological features recently described as significantly more prevalent in basal-like breast carcinomas. Immunohistochemistry revealed expression of basal markers in all cases and the typical basal-like phenotype (ie, ER, PgR, Erbb2 negative and positivity for basal keratins and/or EGFR) in all but three tumours.

Conclusions: We have generated a mouse model of cancer, which mimics the human disease and should prove useful in testing novel and targeted therapies for basal-like breast cancer.

193 The Differential Regulation of p16 and Human Telomerase Reverse Transcriptase May Contribute to the Clinically More Aggressive Behavior of Breast Carcinomas with the Basal-Like Phenotype

A Ribeiro-Silva, S Zucoloto. Ribeirão Preto School of Medicine, Ribeirão Preto, São Paulo, Brazil.

Background: Many independent studies pointed to the existence of a subset of sporadic breast carcinomas with a distinct gene expression signature that includes a high expression of "basal" cytokeratins (CKs) 5 and 17. Breast cancer tumorigenesis is considered to be the result of a multistep process in which the accumulative effect of successive genetics alterations leads to a gradual transition from normal to frankly malignant tissue. This study was carried out to verify the relationship between CK5 expression and several clinicopathologic features and tumor markers of clinical significance in breast pathology.

Design: Immunohistochemical staining (c-erbB-2, c-erbB-3, Chk2, Cyclin D1, CK5, estrogen receptor, Ki67, osteopontin, p16, p21, p27, p53, progesterone receptor, and human telomerase reverse transcriptase) was performed in 100 paraffin sections of invasive primary breast carcinomas and correlated with the patients' clinicopathological characteristics. The following data were retrieved from medical files: age, menstrual status, pathological grading, tumor size, and lymph node metastasis. Statistical analysis was performed using the Graph Pad Prism v.4 software (San Diego, CA).

Results: The positive immunostaining for c-erbB-2, c-erbB-3, Chk2, Cyclin D1, CK5, ER, osteopontin, p16, p21, p27, p53, PR, and telomerase was noted in 27, 60, 77, 41, 19, 53, 65, 44, 25, 38, 35, 46, and 49% of tumors, respectively. In 30 carcinomas, 10 to 50% of neoplastic cells were positive for Ki67, while 17 carcinomas expressed Ki67 in more than 50% of the neoplastic cells. CK5 expression correlated with precocious age ($p=0.0022$), high histologic grade ($p<0.0001$), lymph node metastases ($p=0.0002$), advanced pathologic staging ($p=0.0349$), negativity for estrogen and progesterone receptor ($p<0.0001$), and high Ki67 labeling index ($p=0.0011$). CK5 expression also correlated with p16 ($p=.0215$) and the telomerase ($p=.0047$). The relationship between CK5 expression and the other tumor markers did not reach statistical significance.

Conclusions: In accordance with current literature, CK5 expression correlated with several indicators of aggressiveness. In breast carcinomas, both p16 and telomerase are associated with cellular proliferation and metastases. In that way, the differential expression of p16 and telomerase in breast carcinomas with the basal-like phenotype may contribute to the clinical more aggressive behavior of these tumors.

194 Significance and Relationship between Cytokeratins, p63, β-Catenin and Matrix Metalloproteinases Expression in DMBA-Induced Mammary Tumors in Mice Fed with Fat Diet

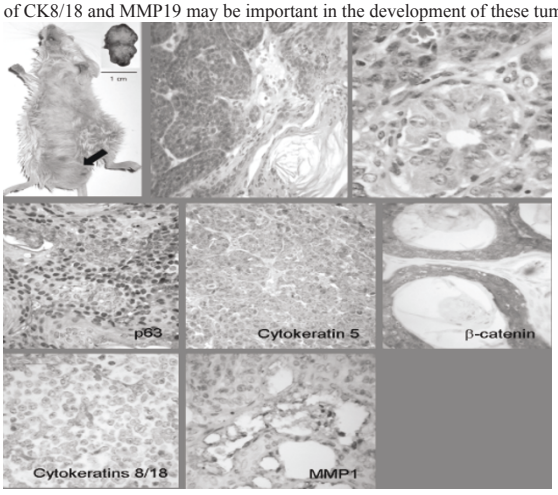
A Ribeiro-Silva, LNZ Ramalho, FS Ramalho, SB Garcia, S Zucoloto. Ribeirão Preto School of Medicine, Ribeirão Preto, São Paulo, Brazil.

Background: The addition of linoleic acid to the diet in mammary carcinomas experimentally induced by 7,12-Dimethylbenz(a)anthracene (DMBA) induces neoplasms with extensive squamous differentiation. This study was carried out to verify the expression of p63, cytokeratin 5 (CK5), cytokeratins 8/18 (CK8/18), β-catenin, and metalloproteinases (MMPs) 1, 2 and 19, in DMBA-induced mammary tumors in mice fed a diet enriched with linoleic acid.

Design: Sixty 8-week-old virgin female balb-c mice were given by gavage 1 mg of 7,12 dimethylbenz[a]anthracecene (DMBA) dissolved in 1 ml of corn oil once a week for 6 weeks. Linoleic acid was supplied as 15 g of corn oil for each 100 g of standard diet. When tumors reached 1 cm in size, euthanasia was performed and mammary tissue was pooled for analysis. Formalin-fixed paraffin-embedded tumors were submitted to immunohistochemical study with CK5, p63, CK8/18, β-catenin, and MMPs 1, 2 and 19.

Results: Fourteen animals developed carcinomas with areas of squamous differentiation which were diffusely positive for CK5, p63, β-catenin, MMP1, and MMP2; and focally positive for CK8/18 and MMP19. Both CK5 and p63 stained the poorly differentiated areas as well as the foci of squamous differentiation.

Conclusions: Our data indicate that the expression of MMP1 and MMP2, the p63-driven nuclear accumulation of beta-catenin, as well as the down regulation of CK8/18, might be important in the tumorigenesis of mammary tumors induced by DMBA in mice fed with fat diet. Unlike most matrix metalloproteinases, MMP19 is downregulated during malignant transformation and histologic dedifferentiation. In this study, MMP19 expression was inversely correlated with p63 and CK5 expression suggesting that progression towards an invasive phenotype may lead to neoplastic dedifferentiation. In conclusion, the overexpression of β-catenin, MMP1 and MMP2 and the down regulation of CK8/18 and MMP19 may be important in the development of these tumors.



195 Accuracy of Intraoperative Sentinel Lymph Node Evaluation for Breast Cancer

V Richards, DF Roses, DM Axelrod, AA Guth, RL Shapiro, J Cangiarella, N Ziguridis, F Darvishian, B Singh. New York University, New York, NY.

Background: Sentinel lymph node assessment has become the standard of care of axillary lymph node assessment in breast carcinoma. The accuracy of intraoperative sentinel lymph node assessment remains controversial and the ASCO guideline recommendations do not clearly endorse intraoperative assessment.

Design: 1500 sentinel lymph nodes from 693 patients at New York University were submitted for intraoperative frozen section and imprint cytology. The duration of intraoperative evaluation was measured from receipt of the first sentinel node to the transmission of the diagnosis to the surgical team. Each lymph node was bisected, imprinted and frozen on one mold. One slide with imprint cytology and two sections (5) of each mold were examined and a diagnosis rendered intraoperatively. For each lymph node three permanent sections(5), 20apart, and immunohistochemical stain for cytokeratin (AE1/AE3) were examined.

Results: Frozen sections accurately diagnosed 556 patients with 1273 lymph nodes with no metastases and 94 patients with 101 lymph nodes with metastases in sentinel lymph nodes (Table 1). Forty three patients with 73 lymph nodes had a negative frozen section, however metastases were detected on permanent sections, with an average size of 1.3 mm. No false positive diagnosis was rendered intraoperatively. The specificity of frozen section is 99.99% and sensitivity is 68.5%. The sensitivity of touch preparation is 58.7% and specificity is 99.99% in a retrospective blinded analysis by a cytopathologist. The positive and negative predictive values for frozen sections are 99.5% and 92.8% respectively. The average time for intraoperative diagnosis was 19 minutes in 50 patients.

Conclusions: In this cohort, frozen sections of sentinel lymph nodes have a very high positive and negative predictive value. The average size of the metastatic focus in false negative lymph nodes was 1.3mm. Based on intraoperative assessment 94 patients had a concurrent axillary dissection, thereby preventing a second surgical procedure. Frozen section assessment of sentinel lymph nodes is credible with an acceptable turn around time for optimal surgical management.

Permanent Sections	Frozen Section/Case		Frozen Section/Lymph Node	
	-	+	-	+
-	556	0	1273	0
+	43	94	73	101

Concordance between Frozen and Permanent Sections

196 Prognostic Markers in Estrogen, Progesterone and Her-2/neu Negative Invasive Breast Carcinomas

RS Ricketts-Loriaux, CN Otis, E Resetkova, N Sneige. M.D. Anderson Cancer Center, Houston, TX; Baystate Medical Center, Springfield, MA.

Background: Triple negative invasive breast carcinomas (TNIBC) are high grade tumors that do not express hormone receptors (ER, PR) or HER-2/*neu* and typically express basal cytokeratins, and/or epidermal growth factor receptor (EGFR). Receptor tyrosine kinases, including EGFR, activate the phosphoinositide-3 kinase/protein kinase B (PI3K/pAkt) pathway. Activation of this pathway in breast cancer is associated with an aggressive clinical course. p53 is also highly expressed in TNIBC. Currently we have no reliable prognostic markers for TNIBC. The aim of this study was to evaluate the prognostic value of EGFR, pAkt and p53 immunomarkers in TNIBC.

Design: Using TMA technology on formalin-fixed paraffin embedded tissue, immunohistochemical expression of EGFR (ZYMED, San Francisco CA), pAkt (Cell Signaling Technology Beverly, MA) and p53 (DAKO, Carpenteria, CA) were evaluated in 60 cases of TNIBC (48 invasive ductal carcinomas, 7 metaplastic carcinomas, 4 medullary-like carcinomas and 1 apocrine carcinoma). All cases were retrieved from the surgical pathology files at M.D. Anderson Cancer Center from patients who underwent breast excision between 1/04 - 6/05 with a median follow-up of 34.6 months. Statistical significance was evaluated using chi-square analysis.

Results: Results for all three markers (EGFR, pAkt, p53) were available in 52 cases. Eight patients (15%) developed either local recurrences and/or metastasis (4 cases) or died of disease (4 cases) during an average follow-up of 14 months. pAkt was overexpressed in 38 (73%) cases and it was the only statistically significant marker shown to be associated with worse patient outcome ($p<0.013$). Neither EGFR or p53 showed statistically significant associations with patient outcome. However, the overexpression of EGFR did show a trend toward being associated with disease-free survival. In terms of profiling, cases positive for both EGFR and pAkt constituted the largest subgroup (26/52, 50%); but only 2 of those presented with metastatic disease. In the EGFR-negative and pAkt-positive subgroup (12/52, 23%), 4 patients (33%) developed metastasis or were dead of disease. Interestingly, an EGFR-negative and pAKT and p53-positive profile (7/52, 13%), showed a propensity to worse clinical outcome (3 deceased, 1 metastases).
Conclusions: Lack of EGFR expression in conjunction with pAkt overexpression in TNIBC may predict a worse clinical course.

197 Rabbit Monoclonal Antibodies Are Highly Cost Effective and a Reliable Alternative to Mouse Antibodies for Estrogen and Progesterone Receptor Evaluation of Breast Cancers by Immunohistochemistry

RM Rocha, CB Nunes, GFS Rocha, FSF Sanches, FN Oliveira, H Gobbi. Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
Background: Immunohistochemical analysis (IHC) for estrogen and progesterone receptors (ER and PR) in breast cancer is a well-established predictive factor for anti-estrogen therapy. However, there is a large and variable choice of antibodies and no recommendation about the best antibodies to be used in clinical and research testing. A novel generation of rabbit monoclonal antibodies (RabMab) has been released recently. In this study, we compared the novel RabMab anti-ER and anti-PR antibodies to the mouse monoclonal antibodies (Mab) using a tissue microarray.
Design: Two cylinders (2mm diameter) of formalin-fixed paraffin embedded tissue were obtained from 24 invasive breast cancers and immunostained using anti-ER Mab (1D5 and 6F11) and RabMab (SP1 and B644), and anti-PR Mab (PgR312 and PgR636) and RabMab (SP2 and B645). The IHC was evaluated considering positive those tumors in which more than 10% of the tumor cell nuclei stained. We also compared the antibody prices and semiquantitatively evaluated staining intensity and background.
Results: Our results showed that both RabMab against ER from two different suppliers presented significantly higher staining intensity and lower cost as compared to the clone 1D5 from three different suppliers and similar intensity as compared to clone 6F11. Both RabMab against PR also showed higher staining intensity as compared to both Mabs and lower cost as compared to Mab PgR636. A sharp staining with low background was observed in the RabMab sections.
Conclusions: The novel RabMab are highly cost effective, reliable and represent a better alternative for ER and PR testing by IHC in formalin-fixed paraffin embedded tissue.

198 *MYC* Amplification in Breast Cancer: A Chromogenic In Situ Hybridisation Study

SM Rodriguez Pinilla, RL Jones, MB Lambros, E Arriola, K Savage, M James, SE Pinder, JS Reis-Filho. ICR, London, United Kingdom; Addenbrooke's Hospital, Cambridge, United Kingdom.
Background: Conflicting results on *MYC* amplification in breast cancer have been reported, with frequencies varying from 1 to 94%, depending on the methodology and cohort studied. Although *MYC* amplification has been linked with poor outcome, it remains unclear whether *MYC* is an independent prognostic factor. In addition, tumours arising in BRCA1 germ line mutation carriers are reported to have a significantly higher prevalence of *MYC* amplification than sporadic breast tumours. The first aim of this study was to analyse the correlation between *MYC* amplification and various clinico-pathological features and outcome in a cohort of 245 patients with invasive breast carcinoma treated with surgery followed by anthracycline-based chemotherapy. Secondly, given the high proportion of *MYC* amplification in tumours of *BRCA1* mutation carriers, we sought to define the prevalence of *MYC* amplification in 'basal-like' breast carcinomas.
Design: *MYC* gene copy number was assessed on a tissue microarray containing duplicate cores of 245 invasive breast carcinomas by means of chromogenic *in situ* hybridisation using SpotLight C-MYC amplification probe and SpotLight Chromosome 8 Centromeric probe (CEP8). Signals were evaluated at 400x magnification; 30 morphologically unequivocal neoplastic cells in each core were counted for the presence of the gene and CEP8 probe signals. Amplification was defined as a MYC: CEP8 ratio >2.
Results: Signals for both *MYC* and CEP8 were assessable in 196/ 245 (80%) tumours. *MYC* amplification was found in 19/ 196 cases (9.7%) and was not associated with tumour size, histological grade, positivity for oestrogen receptor, progesterone receptor, HER2, epidermal growth factor, cytokeratins 14, 5/6 and 17, MIB1 or p53. Only 4% of basal-like carcinomas showed *MYC* amplification, compared to 8.75% and 10.7% of luminal and HER2 tumours. On univariate analysis, *MYC* amplification displayed a significant association with shorter metastasis-free and overall survival and proved to be an independent prognostic factor on multivariate survival analysis.
Conclusions: Although *MYC* amplification is heterogeneous in breast carcinomas, it can be reliably assessed on tissue microarrays containing replicate cores. *MYC* amplification has proven to be an independent prognostic factor for MFS and OS.

199 Sporadic Basal-Like Breast Carcinomas Harbour *BRCA1* Gene Promoter Methylation and sox2 Overexpression: Similarities with *BRCA1*-Germ Line Mutated Tumours

SM Rodriguez-Pinilla, D Sarrio, G Moreno-Bueno, Y Rodriguez-Gil, MA Martinez, L Hernandez, D Hardisson, JS Reis-Filho, J Palacios. CNIO, Madrid, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital La Paz, Madrid, Spain; Institute of Cancer Research, London, United Kingdom; HHUU Virgen del Rocio, Sevilla, Spain.
Background: Tumours arising in *BRCA1* mutation carriers and sporadic basal-like breast carcinomas have phenotypic, immunohistochemical and clinical similar charactericts. Since *BRCA1* mutations are rarely found in sporadic tumours other epigenetic or genetic mechanisms of BRCA1 inactivation and alterations in other genes implicated in the same pathways have been suggested. *SOX2* is an embryonic transcriptor factor located at chromosome 3q, a region frequently gained in *BRCA1*-germline mutated tumours. The aim of the study was to establish whether *BRCA1* and/or *FANCF* promoter methylation, *BRCA1* LOH and sox2 expression were related to basal-like sporadic breast tumours.
Design: 115 sporadic grade 3 tumours including 32 atypical medullary carcinomas (AMC) were immunohistochemically analysed for oestrogen receptor (ER), progesterone receptor (PR), CK8, CK19, CK14, CK5/6, EGFR, HER2, ki67, p53, p-cadherin, vimentin, p63, calponin, caldesmon, CD10, smooth muscle actin, S-100 protein, cdk1, cdk2, cyclin E, cyclin A, cyclin D1, cyclin B1, Rb, survivin, topoisomerase II alpha, p21 and p27 using tissue microarrays. Unsupervised hierarchical cluster was used to defined tumours having basal-like phenotype.
Results: 69 cases (60%) displayed a basal-like phenotype, 31 atypical medullary carcinomas (AMC) and 38 ductal carcinomas. *BRCA1 was methylated* in 45.7% (27/59) of cases and showed a statistically significant correlation with basal-like phenotype ($p=0.028$), while *FANCF* methylation was found in 3 out of 50 cases (2 basal-like and one non-basal-like carcinomas). Sox2 expression was observed in 26.6% of cases and was significantly more frequently expressed in basal-like breast carcinomas ($p<0.001$).
Conclusions: Our results demonstrate that sporadic basal-like carcinomas frequently harbour *BRCA1* gene promoter methylation and share phenotypic characteristics with tumours arising in *BRCA1* germline mutation carriers. Sox2 is prefentially expressed in tumours with basal-like phenotype and may play a role in defining their less differentiated/'stem cell' phenotypic characteristics.

200 Truncated HER2 Receptor in Breast Cancer Correlates with Poor Prognosis and Trastuzumab Resistance

F Rojo, S Landolfi, J Lirola, J Jimenez, S Rodriguez, E Llonch, J Baselga, S Ramon y Cajal. Vall d'Hebron University Hospital, Barcelona, Spain.
Background: Overexpression/amplification of the human epidermal growth factor receptor-2 (HER2) occurs in approximately 25% of patients with breast cancer and is associated with aggressive disease and decreased survival. HER2 is a receptor tyrosine kinase that belongs to the Epidermal Growth Factor Receptors (EGFRs or HERs). The monoclonal antibody trastuzumab binds to the extracellular full length HER2 receptor and has shown to improve survival in advanced breast, and more recently, in early breast cancer as well. Unfortunately, not all patients respond to trastuzumab or those patients that respond initially will eventually progress due to secondary resistance. HER2 C-Terminal Fragments (CTFs), assumed to encompass the transmembrane and cytoplasmic domain of HER2, are frequently found in human breast tumors. Despite the functional potential and the clinical importance of these CTFs, which associate with nodal metastasis and poor prognosis CTFs lack the transmembrane domain but conserve the whole kinase domain and might be involved in the sensitivity to trastuzumb therapy.
Design: An immunofluorescence double-staining assay to specifically detect HER2 CTFs using antibodies against the extracellular and intracellular domains of HER2 (CBE1 and CB11, respectively) and anti-cytoplasmic markers has been tested in a series of 43 paraffin-embedded breast tumors from patients treated with trastuzumab. Using a digital analysis device, co-localization between both antibodies will indicate the presence of full-length HER2, while immunoreactivity against CB11 alone will show the presence of CTFs.
Results: Out of these 43 patients, 8 were positive for HER2 CTFs expression and the remaining 48 tumors expressed only full length HER2. HER2 CTFs expression strongly correlated with trastuzumab resistance. Only 1 of the 8 patients positive for HER2 CTFs responded to trastuzumab while 18 of the 35 patients without HER2 CTFs had a response (response rate 12.5%) (Chi square test, $p=0.045$). Moreover, presence of HER2 CTFs correlated with poor survival compared with patients with full length receptor expression (Kaplan-Meier survival, $p=0.0192$).
Conclusions: In HER2 overexpressing breast cancer, presence of C-terminal truncated forms of the receptor confers a poor prognosis and might be a mechanism of sensitivity to trastuzumab therapy. These CTFs can be detected with double immunofluorescence assays on paraffin-embedded clinical samples.

201 Histology of Breast Re-Excision Specimens Following Intraoperative Radiotherapy

ME Roth, CI Sartor, CA Livasy. University of North Carolina, Chapel Hill, NC; Univerisity of North Carolina, Chapel Hill, NC.
Background: Intraoperative radiotherapy (IORT) is a technique employed in breast conserving surgery where a single dose of radiation is delivered to the surgical cavity at the time of excisional biopsy. Since most in-breast recurrences occur in the lumpectomy bed, the objective is the sterilization of residual cancer cells in the operative area in a single radiation dose. Re-excision or mastectomy is occasionally recommended following breast conserving surgery and IORT. In this study we report on the potential limitations of evaluating the histology of normal and neoplastic breast following IORT.

Design: Women involved in a phase II trial of single-dose IORT were identified at our institution between 2003 and August, 2006. Each woman received a single dose of 13-17 Gy to the tumor bed at the time of initial segmental mastectomy. Patients who underwent subsequent breast re-excision due to the presence of carcinoma at or near the original resection margin were identified. Hematoxylin and eosin stained slides from each of the re-excision specimens were reviewed in detail to evaluate for changes in the normal and, if present, neoplastic breast tissue.

Results: Fifty-five women with a median age of 63 received IORT at the time of breast conserving therapy. The median clinical tumor size as defined by ultrasound was 1.1 cm, with a range of 0.5-2.1 cm. Thirteen (24%) women required subsequent re-excision of close or positive margins. Re-excisions were performed on average 21 days following the primary surgery. Eight of 13 (61%) re-excision cases contained regions of diffuse coagulative necrosis adjacent to the biopsy cavity that limited histologic evaluation for residual invasive or in situ disease. Cellular intraductal processes could not be accurately categorized in these cases. Two of the 13 cases contained an infiltrative pattern of necrotic ghost cells suspicious for treated invasive carcinoma. Accurate tumor measurement and margin assessment were limited in these 2 cases. The viable regions in the re-excision specimens showed reactive atypia within stromal fibroblasts and focal ductal and lobular cytologic atypia, but these findings did not limit interpretation.

Conclusions: IORT for invasive breast carcinoma may induce geographic zones of coagulative necrosis of breast tissue adjacent to the biopsy cavity limiting histologic evaluation for residual invasive and in situ carcinoma in re-excision specimens. These changes may have implications for margin and AJCC pathologic stage assessment.

202 Overexpression of Cyclin D1 Predicts Poor Response to Neoadjuvant Chemotherapy in T2-T4 Breast Cancer Patients

RS Saad, KL Denning, M Valenzu, T Julian, YL Liu, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.

Background: The amplification of cyclin D1 has been found to be associated with decreased relapse-free and overall survival. However, its clinical significance as a prognostic marker remains unclear, similar to epidermal growth factor receptor (EGFR) and MIB-1. The predictive value of these markers prior to neoadjuvant treatment has not been well studied. We evaluated cyclin D1, EGFR and MIB expression in T2-T4 breast carcinomas, and their relationship with clinical-pathological response to neoadjuvant chemotherapy.

Design: We identified 85 patients who presented with large breast masses (T2-T4) to our breast clinic. Core needle biopsies were available in 45 patients for immunohistochemical studies. The patients were then treated with four-eight cycles of doxorubicin/docetaxel and occasionally Taxotere (17 patients), followed by partial mastectomy (27/45, 60%) or total mastectomy (18/45, 40%) and lymph node dissections. Complete pathologic response was defined as absence of invasive carcinoma at resection, while incomplete pathologic response was defined as having any invasive carcinoma. Cases were immunostained for cyclin D1, EGFR and MIB-1.

Results: Overexpression of cyclin D1 was found in 58% (26/45) of the cases and correlated with estrogen receptor (ER) expression ($p < 0.01$). EGFR was positive in 16/45 (36%) of the cases which was expressed in the cytoplasm of the cancer cells, with occasional cell membranes staining. MIB-1 (>20%) was expressed in 15/45 (33%) of the cases. There were significant association between overexpression of cyclin D1 and poor clinical and pathologic response. Overexpression of cyclin D1 has significant adverse effect on overall survival and relapse-free survival ($p = 0.01$). Overall, cyclin D1 was not associated with other clinicopathological features. EGFR was significantly expressed in ER and PR negative tumors (r=0.36 and 0.7, respectively). MIB-1 showed significant association with nuclear grade and lymph node metastases (r=0.66 and 0.32), respectively. Both EGFR and MIB-1 showed no significant correlation with pathologic response or patient survival.

Conclusions: Our findings indicate that overexpression of cyclin D1 correlates with poor response to neoadjuvant chemotherapy and a worse prognosis in T2-T4 breast cancer patients. MIB-1 may have prognostic significance by correlating with nuclear grade and lymph node status, but EGFR provides no additional prognostic information.

203 Validation of Rabbit Monoclonal Antibodies (ER/PR/Her-2, MIB-1) in Breast Carcinoma

Z Salih, C Cohen, A Nassar. Emory University, School of Medicine, Atlanta, GA.

Background: The determination of estrogen (ER) and progesterone (PR) receptor status has become standard practice in the evaluation of patients with invasive breast cancer, having important prognostic and therapeutic implications. Her-2 assessment is important to evaluate the response to Herceptin® (Trastuzumab) therapy for primary and metastatic breast cancer. MIB-1 labeling index is a reliable, practical, and useful method of measuring proliferative activity in invasive breast cancer, and is an important predictor of clinical behavior. This study is undertaken to compare rabbit monoclonal antibodies (rmab) for ER, PR, Her-2 and MIB-1 against FDA-approved monoclonal and polyclonal antibodies (mpab) as the gold standard.

Design: A tissue microarray of a Mexican cohort of 43 breast cancer patients was used. Immunohistochemistry was performed, following optimized epitope retrieval, with a polymer based detection system (Envision-plus, DakoCytomation) using rmab (Lab Vision): ER (SP1; 1:100), PR (SP2; 1:400), Her-2 (SP3; 1/100) and MIB-1 (SP6; 1:200). FDA approved mpab (Dako) used were: ER (1D5; 1/50); PR (PgR636; 1/400); Herceptin kit according to manufacturer's instructions, and MIB-1 (Mib-1; 1/160). Scoring was performed using <10% as negative for ER and PR, 0 and 1+ as negative for Her-2. For MIB-1, all cases were positive, divided into low (<10%); intermediate (11-25%), and high (>50%) positivity.

Conclusions: The positivity rate for rmab SP clones is higher than their FDA-approved ER and PR, statistically significant for PR but not ER. Her-2 (FDA-approved Herceptin) positivity rate is statistically significantly higher than the rmab. The rmab for ER (SP1) and PR (SP2) are more sensitive than the FDA-approved clones, but PR is less specific. Her-2 (SP3), on the other hand, is more specific, but less sensitive, than Herceptin.

Positivity rate of rmab and FDA-approved antibody clones

	rmab (%)	FDA mpab (%)	p-value
ER (positivity rate)	67.5	58.1	0.496
PR (positivity rate)	100	61.9	0.0001
Her-2 positivity rate	18.6	44.4	0.011
MIB-1 proliferation			0.608
Low	55.6	44.2	
Intermediate	19.4	23.3	
High	25.0	32.6	

Accuracy, Sensitivity, Specificity of rmab clones using FDA approved antibodies as gold standard

	Accuracy (%)	Sensitivity (%)	Specificity (%)	p-value
ER (rmab)	92.5 (37/40)	100	81.3	0.125
PR (rmab)	63.2 (24/38)	100	0	0.0001
Her-2 (rmab)	68.5 (24/35)	23.8	86.3	0.002
MIB-1 (rmab)	63.2 (24/38)			

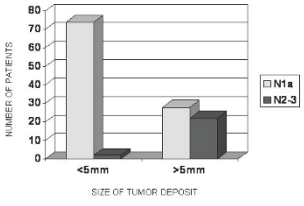
204 Size of Sentinel Node Tumor Deposits and Number of Positive Axillary Nodes: Some Breast Cancer Patients May Benefit from Less Aggressive Axillary Dissections

E Samoilova, JT Davis, YM Brill, M Cibull, P McGrath, E Romond, A Moore, LM Samayoa. University of Kentucky, Lexington, KY; VA Hospital, Lexington, KY.

Background: Approximately 30% of all breast cancer patients with clinically negative (-) axillae are Sentinel Node (SN) positive (+). In 60% of these patients the SN is the only (+) node after a complete axillary dissection (AD). The object of this review was to identify a subset of patients who might benefit from a less aggressive AD, thereby reducing complications without compromising staging accuracy and local disease control.

Design: Records of 467 patients with clinically (-) axillae who underwent SN mapping were reviewed. Primary Tumor (PT) characteristics of all SN (+) patients were recorded: size of PT, grade, presence of scar, stromal retraction artifact (RA), lymph-vascular invasion (LVI), hormone receptor and HER2/neu status, as well as size of SN, size of tumor deposits, presence of RA in SN, SN extra-capsular extension (ECE), number (#) of SN and Non Sentinel Nodes (NSN), and # of (+) SN and NSN. Statistical analysis was performed using chi-square test.

Results: 70% of the patients (n = 327) were SN (-), 27% (n = 126) had (+) SN and 3% (n = 14) were mapping failures. Of the 126 SN (+) patients, 81% (n = 102) had a maximum of 3 (+) LN (N1a), 78% (n = 80) of which were SN (+) only. Of the latter, 86% (n = 69) had a single (+) LN. 5% (n = 24) of all patients undergoing SN mapping had >3 (+) nodes (N2-3). Although presence of LVI in the PT and ECE in the SN were significantly different between N1a and N2-3 patients ($p < 0.05$ and $p < 0.01$, respectively), the only independent variable was the size of the SN tumor deposit ($p < 0.001$). Patients with SN tumor deposits 5 mm or less have a 97% chance of having 3 or less (+) LN, the majority of them (86%) with a single (+) LN.



Out of 75 patients that had SN tumor deposits measuring 5 mm or less, 97 % (n = 73) were N1a. In the group of 51 patients with SN tumor deposits exceeding 5 mm, 57 % (n = 29) were N1a and 43 % (n = 22) were N2-3.

Conclusions: Extensive axillary dissection may not be required in patients with SN tumor deposits measuring 5mm or less; however, a more complete axillary dissection remains indicated for patients with SN tumor deposits measuring more than 5mm.

205 Tumor Type, Nottingham Grade, and Survival Following a Diagnosis of Atypical Ductal Hyperplasia on Initial Breast Biopsy

ME Sanders, PA Schuyler, JF Simpson, WD Dupont, DL Page. Vanderbilt University Medical Center, Nashville, TN.

Background: Atypical ductal hyperplasia (ADH) is associated with a 10-20% absolute risk of subsequent invasive carcinoma in either breast. We have recently reported a significant proportion of invasive breast cancers (IBC) following a diagnosis of atypical lobular hyperplasia (ALH) to be special type cancers of low Nottingham grade with excellent long-term survival.

Design: A longitudinal follow-up study was conducted of 241 women who underwent 245 benign surgical biopsies between 1950 and 1988 with a diagnosis of ADH. Subsequent IBCs were graded using the Notttingham criteria and subtyped based on histologic features. Survival after cancer diagnosis was ascertained.

Results: 222 women had a diagnosis of ADH alone while 19 women were diagnosed with ADH and ALH. Thirty eight (16%) of these women developed IBC. Thirty-one of these cancers were in women with ADH alone and 7 were in women with ADH and ALH. 14% (31/222) of women with ADH alone developed IBC where as 37% (7/19) of women with ADH and ALH developed IBC. By an average of 12 years after invasive cancer diagnosis, only 17% (1/6) of women with special type or variant tumors died of breast cancer, compared with 25% of women (8) with tumors of no special type (n=25) or an unknown type (n=7). Only one patient with a tumor of low Nottingham grade died of breast cancer. All breast cancer deaths occurred in women who had ADH only in their initial breast biopsy.

Conclusions: ADH is a non-obligate cancer precursor associated with a moderate risk of breast cancer similar to ALH. Although later cancers are less likely to be of special type following a diagnosis of ADH, the majority are of low or intermediate combined histologic grade with a good prognosis. While women with ALH and ADH developed cancer twice as often as women with ADH alone there were no cancer-related deaths in this group suggesting that the combination does not indicate a worse prognosis.

206 Caveolin 1 Is Overexpressed and Amplified in a Subset of Basal-Like and Metaplastic Breast Carcinomas: A Morphological, Ultrastructural, Immunohistochemical and *In Situ* Hybridisation Analysis

K Savage, MBK Lambros, D Robertson, RL Jones, C Jones, A Mackay, M James, JL Hornick, EM Pereira, F Milanezi, CDM Fletcher, FC Schmitt, A Ashworth, JS Reis-Filho. ICR, London, United Kingdom; ICR, Sutton, United Kingdom; Harvard Medical School, Boston, MA; Sao Paulo, Brazil; Porto, Portugal.

Background: Caveolin1 is a major component of caveolae, whose distribution and significance in different breast cell types and role in breast carcinogenesis remain poorly understood. Both tumour suppressive and oncogenic roles have been proposed for this protein.

Design: The aims of this study were three-fold: to characterise the distribution of caveolin1 in normal human breast tissue, benign breast lesions, breast cancer precursors and metaplastic breast carcinomas, tumours with basal/ myoepithelial differentiation; to assess the prognostic significance of caveolin1 expression in a cohort of 245 invasive breast carcinomas; and to define whether *CAV1* gene amplification is the underlying genetic mechanism driving caveolin1 overexpression in breast carcinomas. The distribution of caveolin1 in frozen and paraffin-embedded whole tissue sections of normal breast, benign breast lesions, breast cancer precursors and invasive breast cancers was evaluated using immunohistochemistry, immunofluorescence and immuno-electron microscopy. In 25 cases *CAV1* gene amplification was assessed by chromogenic *in situ* hybridisation, using in-house generated probes.

Results: Caveolin1 was consistently expressed in myoepithelial cells, endothelial cells and a subset of fibroblasts. Luminal epithelial cells showed negligible staining. Caveolin1 was expressed in 90% of 39 metaplastic breast carcinomas and in 9.4% of 245 invasive breast cancers from patients treated surgically followed by anthracycline-based chemotherapy. In the later cohort, caveolin1 expression was significantly associated with 'basal-like' immunophenotype and with shorter disease-free and overall survival on univariate analysis. Two of 15 cases (13%) with strong caveolin1 expression displayed *CAV1* gene amplification.

Conclusions: Our results confirm the preferential expression of caveolin1 in myoepithelial cells and tumours with myoepithelial/ 'basal-like' phenotype. The finding of concurrent *CAV1* amplification and overexpression calls into question their tumour suppressive effects in 'basal-like' breast carcinomas.

207 Expression of the Cell Adhesion Molecule Laminin-5 Correlates with p63 Expression in Metaplastic Carcinoma of the Breast

CL Schiller, WR Wiseman, SM Sitterding, C Luan, WG Watkin, EL Wiley, MZ Gilcrease, LK Diaz. Feinberg School of Medicine Northwestern University, Chicago, IL; Evanston Northwestern Healthcare, Evanston, IL; University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The developmental and stem cell regulatory protein p63 is frequently expressed in metaplastic carcinomas of the breast. Metaplastic carcinomas belong within the basal-like group of breast cancer although specific molecular alterations underlying the morphologic phenotypes that characterize metaplastic differentiation are not known. p63 was recently shown to regulate gene expression of laminin-5 (LN5) and α6β4 integrin in a breast cell line model. These cell adhesion molecules are known to play important roles in tumor cell migration and invasion and frequent expression of LN5 and α6β4 integrin in basal-like and metaplastic breast carcinomas have been observed. For the current study, we sought to verify these recent cell line findings in a cohort of basal-like and metaplastic breast tumors. Our goal was to determine if co-expression of p63 and either of these cell adhesion proteins could be observed.

Design: A total of 54 cases were identified including 27 metaplastic and 27 basal-like (non-metaplastic) carcinomas. Immunohistochemical detection of LN5 and α6β4 integrin was performed using monoclonal antibodies and standard automated procedures. For p63 staining a manual methodology was used. The cutoff for p63 positivity was 10% or greater nuclear staining. The results were compared using a Chi-square statistical calculation.

Results: For all 54 cases studied, LN5 expression was found to correlate significantly with expression of p63 (χ^2=7.2; p≤.01). When the metaplastic and basal-like groups were analyzed separately, the metaplastic group showed the strongest correlation between LN5 and p63 (χ^2=8.6; p≤.01). 10 of 12 metaplastic carcinomas found to be positive for p63 also expressed laminin-5. All 3 basal-like carcinomas positive for p63 also expressed LN5. No significant correlations between α6β4 integrin and p63 were observed for any category.

Conclusions: As p63 is known to regulate laminin-5 expression in vitro, frequent coordinate expression of LN5 and p63 in metaplastic and basal-like carcinomas suggests that laminin-5 expression may be regulated by p63 in these breast cancer subtypes. Upregulation of laminin-5 by p63 may be one of the molecular mechanisms responsible for the unique phenotype and aggressive behavior of these tumors.

208 Surgical Follow up of Benign Intraductal Papillomas Diagnosed by Stereotactic Ultrasound Guided Breast Biopsy

MJ Schniederjan, GM Oprea, MB Mosunjac. Emory, Atlanta, GA.

Background: Ultrasound guided stereotactic core needle biopsy (CNB) is increasingly being utilized in the diagnosis of nonpalpable breast lesions. At the present time extensive reports are available regarding the management of atypical papillary lesions, however the significance of a diagnosis of benign intraductal papilloma (IDP) in these specimens is controversial. Only a few small series exist which suggest a small risk of carcinoma.To further investigate such risk, we evaluated a large series of 82 CNB containing benign IDP that were followed by excisional biopsy.

Design: Retrospective review of all CNB containing the diagnosis of benign intraductal papilloma in the period from 2004-2006 were extracted and cases with subsequent excisional biopsy were further analyzed. Cases were divided in following groups: intraductal papilloma only (IDP), intraductal papilloma with adjacent usual ductal hyperplasia (IDP+UDH), intraductal papilloma and adjacent atypical ductal hyperplasia (IDP +ADH), intraductal papilloma and adjacent atypical lobular hyperplasia (IDP +ALH) and intraductal papilloma with adjacent DCIS (IDP+ DCIS). Diagnoses were compared to the follow-up excisional biopsies to asses the potential risk of developing a subsequent DCIS and/or infiltrating carcinoma.

Results: There were 168 cases with initial diagnosis of IDP on CNB, out of which 82 cases (48.8%) were followed by excisional biopsy. The patients age ranged from 28-75 years (median = 58 years) and there were 41 AA, 31 Caucasian, 3 Asian, 1 Hispanic and 6 racially unclassified patients. In the IDP group 7.1% of cases (2/28), contained DCIS in the follow up excisional biopsy, with no invasive carcinomas identified. IDP+UDH group showed progression to infiltrating carcinoma in three cases (3/28, 10.7%), and to DCIS in 2 cases (2/28, 7.1%). In IDP +ADH group one case was upgraded to DCIS (1/12, 8.3%). In IDP+ALH group (2cases) no significant change in diagnosis was noted. Five cases initially diagnosed as IDP+DCIS were downgraded to ADH on excisional biopsy (5/12, 41.7%), whereas none of the cases initially diagnosed with IDP + DCIS (0/12) showed infiltrating carcinoma at excision.

Conclusions: The results show that a significant proportion of benign papillary lesions identified at core needle biopsy were subsequently associated with ADH, DCIS or invasive carcinoma upon examination of the following excision specimen. This finding supports the current recommendation for excision of all papillary lesions.

209 Malignant Spindle Cell Tumors of the Breast Demonstrate Overlapping Immunoprofiles for Cytokeratin and Myoepithelial Markers

SJ Shin, PP Rosen. Weill Medical College of Cornell University, New York, NY.

Background: Morphologic features of malignant mammary spindle cell tumors often overlap and immunohistochemical analysis is heavily relied upon for diagnosis. The current literature suggests there is overlapping immunohistochemical profiles among these tumors. We set out to evaluate the immunoprofile of these tumors using a spectrum of cytokeratin (CK) and myoepithelial (M) markers including recently described CD29 and 14-3-3σ.

Design: 37 malignant spindle cell tumors of the breast were retrieved from our surgical pathology breast consultation files. The histologic diagnoses of spindle cell metaplastic carcinoma (MC) (26), sarcoma (S) (3) and phyllodes, high-grade (P-HG) (3) and low-grade malignant (P-LGM) (5) were confirmed. Immunohistochemical stains for M markers (CD 29, 14-3-3σ, p63) and CKs including basal-like (CK AE1/3, CK7, K903, CK5) were performed on a representative tumor block from each case. Four µ thick sections were stained on the Bond-Max autostainer from Vision BioSystems (Melbourne, Australia) using the Bond Polymer Defined Detection System. Extent (focal, regional, diffuse) and intensity (weak, moderate, strong) of staining were scored in each case. The results were compared.

Results: For MC, CK 5 (73%) and p63 (89%) were the most frequently positive CK and M markers. Six of 26 MC were negative for all CKs and one was negative for all stains except CKAE1/3. Of the five MC where five stains were negative, three were positive for only M markers CD29 and p63. The remaining two demonstrated positivity for one CK and one M stain. All three S demonstrated focal p63 immunoreactivity and was negative for all other stains. One of three P-HG demonstrated stromal positivity for both CD29 and p63. Two of five P-LGM showed weak staining for CD29.

Conclusions: CK5 and K903 are the most frequently expressed CKs in MC. CD29 and p63 are the most sensitive but not specific M markers for MC. In the absence of reactivity for CK, the diagnosis of MC can be suspected but not fully substantiated on the basis of M markers alone.

210 Characterization of Diagnostically Problematic Spindle Cell Lesions Arising in Papillary Tumors of the Breast

SJ Shin, YF Liu. Weill Medical College of Cornell University, New York, NY.

Background: Spindle cell lesions uncommonly arise in pre-existing papillary and adenomyoepithelial tumors of the breast. They are diagnostically problematic because they are morphologically similar yet represent the pathologic spectrum of benign to malignant lesions. We set out to better define the histomorphologic and immunohistochemical features of this unique subgroup of breast lesions.

Design: 29 spindle cell lesions arising in a pre-existing papillary or adenomyoepithelial tumor were retrieved from our breast pathology consultation files. A control group of 26 spindle cell metaplastic carcinomas (SCMC) unassociated with a papillary tumor were additionally retrieved. The histologic diagnoses were confirmed. Immunohistochemical stains for myoepithelial (M) markers (CD29, 14-3-3σ, p63, CD10) and cytokeratins (CK) including basal-like (CK AE1/3, CK7, K903, CK5) were performed on a representative tumor block from each case. Sections were stained on the Bond-Max autostainer from Vision BioSystems (Melbourne, Australia) using the Bond Polymer Defined Detection System.

Results: SCMC represented the most common spindle cell lesion [21/29 (72%)]. This group consisted of 15 SCMC arising in a papilloma (P) or adenomyoepithelioma (AME), 3 SCMC arising in an atypical P or papillary carcinoma, and 3 SCMC arising in a malignant AME. The remaining cases were 4 myoepithelial carcinoma arising in a AME or P, 2 P or AME with biopsy site changes, 1 malignant AME arising in a papillary carcinoma, and 1 atypical myoepithelioma arising in a P. CK 5 (93%) was the most commonly expressed CK while several M markers were consistently positive [CD29 (100%), CD10 (100%) and p63 (92%)] in these SCMC. Biopsy site changes in one P also demonstrated CD10 reactivity. SCMC arising in malignant AME were also consistently positive for other CKs (CK AE1/3, CK7, K903) in addition to CK5. In the control group of SCMC, CK 5 (73%) and p63 (89%) were the most frequently positive CK and M markers, respectively.
Conclusions: SCMC arising in a pre-existing P or AME was the most common histologic finding. These SCMC do not appear to differ morphologically or immunohistochemically from those unassociated with a papillary tumor. Reactive (prior biopsy site) changes represented only a minority of cases causing diagnostic difficulty in this pathologic scenario.

211 Frequency of Basal like Phenotype and αB-Crystallin Expression in Invasive Breast Cancer in Women under 30 Years of Age

SM Sitterding, WR Wiseman, H Hwang, B Susnik, LK Diaz, VL Cryns, MS Rao, CL Schiller. Northwestern Univ. Feinberg School of Medicine, Chicago, IL.
Background: Gene expression studies have elucidated genetically distinct subtypes of breast cancer, including ER+, HER2+ and basal-like groups. Basal-like breast cancer (BLBC), defined as ER-/HER2- and CK5/6+ and/or EGFR+, accounts for approximately 15% of breast cancer overall and exhibits aggressive behavior and poor prognosis. The small heat shock protein αB-crystallin, which is associated with poor clinical outcome in breast cancer, was recently found to be preferentially expressed in the BLBC subtype. Women under 30 are a special population of breast cancer patients that differ from older patients with respect to relative frequencies of familial vs. sporadic carcinomas, hormone receptor status, tumor grade, and cancer phenotype. These patients may also differ in their relative proportions of breast cancer subtypes as compared to older patients. The aim of this study was to examine the frequency of BLBC and the rate of αB-crystallin expression in women under 30.
Design: 14 cases of invasive breast carcinoma (IBC) in patients with initial presentation at <30 years old (range 21-29, avg. 26.7) were retrieved from our files and stained using automated methods with antibodies to ER, PR, HER2, CK5/6, EGFR and αB-crystallin. αB-crystallin was scored as weakly positive if 1-30% of tumor cells were positive and strongly positive if ≥30% of tumor cells were positive. Results were analyzed with respect to the frequency of the BLBC phenotype and rates of expression of αB-crystallin.
Results: 5/14 (36%) cases of IBC in patients <30 demonstrated a BLBC phenotype. 7/14 (50%) cases were ER+, 1/14 (7%) was HER2+ only and 1/14 (7%) was PR+ only. The proportion of BLBC was found to be similar in African Americans (2/5; 40%) and Caucasians (3/9; 33%). αB-crystallin expression was detected in 6/14 cases (43%), including all 5 BLBC cases and the single PR+ case.
Conclusions: These results indicate that BLBC occurs more frequently in patients under 30 than in the population as a whole (36% in this study vs. 15% in the literature). As observed previously for breast cancer in general, αB-crystallin was also found to be highly specific for the BLBC phenotype in this less than 30 age group. These results suggest that the relatively high frequency of BLBC and αB-crystallin expression may contribute to the aggressive behavior and poor prognosis of breast cancer in women under 30.

212 Histologic Changes in Breast Carcinoma after Neo-Adjuvant Chemotherapy Correlate with Pathologic Response

PS Sullivan, SK Apple. UCLA Medical Center, Los Angeles, CA.
Background: Neoadjuvant chemotherapy (NACT) is becoming the standard of care in locally advanced breast cancers. However, there is currently no standardized pathology reporting format for assessing chemotherapy effect in breast carcinoma. Our study examined the post-NACT response between ductal and lobular carcinoma in the primary tumor, lymphatic/vascular space invasion (LVI), lymph node metastases, and DCIS, compared pre- and post-NACT biomarker changes, and correlated the histologic changes to tumor response and patient outcome.
Design: We retrospectively identified 49 cases of invasive breast carcinoma (40 ductal, 9 lobular) and compared pre- and post-NACT specimens. Type of NACT received, Scarff-Bloom-Richardson grade, and pathologic response were assessed. A five-point scoring system based on the presence of apoptosis, necrosis, histiocytes, degenerative nuclei or ballooning cytoplasm in tumor cells was used to evaluate chemotherapy effect. Breast biomarkers were assessed before and after NACT: estrogen receptor, progesterone receptor, HER-2/neu, and p53. Clinical follow-up of two years was obtained for 29 cases.
Results: 28% and 45% of ductal carcinomas had complete and partial pathologic response compared to 0% and 22% of lobular carcinomas. Chemotherapy effect was present in 38-53% of primary ductal carcinomas and in 37-87% of ductal LVI, lymph node metastases, and DCIS. No chemotherapy effect was seen in any lobular primary carcinoma, LVI, lymph node metastases, or DCIS**.** In ductal carcinomas, partial pathologic response correlated with greater chemotherapy effect using the five-point scoring system (p=0.01). One-fourth of ductal carcinoma cases were associated with alterations in breast biomarkers. Of the ductal carcinoma patients, 19 had no evidence of disease (NED), 4 were alive with disease (AWD), and 1 patient died of disease (DOD). 11% of NED patients had a complete response compared to 50% and 100% of AWD and DOD patients. All patients with lobular carcinoma had no evidence of disease.
Conclusions: Chemotherapy effect is not observed in lobular carcinoma. It is present in 29-68% of primary ductal carcinomas, LVI, lymph node metastases, and DCIS. The presence of chemotherapy effect correlates with tumor pathologic response in ductal carcinomas, both of which may be associated with poor survival. The use of NACT is increasing, and pathologists will need to recognize these changes in the treated tissue. We propose pathology reporting of ductal breast carcinoma to include assessment of chemotherapy effect as outlined above.

213 Inference of the Basal Epithelial Phenotype in Breast Carcinoma from Differential Marker Expression, Using Tissue Microarrays

ZM Sussman, C Cohen, P Haun, D Lawson, A Nassar. Emory University, Atlanta, GA.
Background: The basal epithelial phenotype of breast carcinoma, as determined using gene microarray profiling, has been associated with high-grade and metaplastic morphology, uniform negativity for ER, PR, and Her-2, expression of basal-type cytokeratins, and decreased overall survival. Breast carcinoma in young women is also generally high-grade and prognostically unfavorable.
Design: We compare two groups of breast cancers, (i) ER-, PR-, Her2- (triple negative) [n = 56] and (ii) breast cancers occurring in women under 35 [n = 29], using tissue microarrays, to characterize expression of the basal cytokeratins (CK5/6, CK7, CK14), luminal cytokeratins (CK8, CK18, CK19), EgFR, p-cadherin, and p53.
Results: Statistically significant results are summarized in Table1.

Table 1

	triple negative	age under 35	Fisher p-value
mean age	53.2 (25-88)	29.4 (23-35)	
grade III	47/58 (81%)	21/28 (75%)	0.577
> 1 + lymph node	16/41 (39%)	2/17 (71%)	0.043
ER	0/56 (0%)	16/29 (55%)	<0.0001
PR	0/56 (0%)	14/29 (48%)	<0.0001
Her-2	0/56 (0%)	18/18 (100%)	<0.0001
EgFR	46/54 (83%)	6/25 (24%)	<0.0001
CK14	24/54 (44%)	4/26 (15%)	0.013
CK5/6	12/54 (22%)	1/27 (4%)	0.051
CK8	29/48 (60%)	4/26 (92%)	0.006

Results were not statistically ($p > 0.05$) significant for differences in staining for CK7 (49/53 [93%] vs 26/27 [96%]), CK18 (46/49 [94%] vs 26/27 [96%]), CK19 (42/52 [80%] vs 23/25 [92%]), p-cadherin (52/52 [100%] vs 26/26 [100%], and p53 (39/52 [75%] vs 21/27 [78%].
Conclusions: Triple negative breast cancers predict higher levels of basal/ myoepithelial cytokeratins (CK 5/6, CK14) and EgFR expression, and lower expression of the luminal cytokeratin CK8 compared with similar high grade, non-triple-negative tumors occurring in women under 35. This finding supports prediction of the basal epithelial phenotype using triple-negative receptor status, with positive expression of basal cytokeratins and EgFR.

214 Topoisomerase IIa in Invasive Breast Cancer of Older Women Correlates with Tumor Size and Presence of Ductal Carcinoma In Situ: A Tissue Microarray Study

PH Tan, A Somani, BCS Ho, HH Li, PCS Ang. Singapore General Hospital, Singapore, Singapore; National Cancer Centre, Singapore, Singapore.
Background: Topoisomerase IIa is an enzyme linked to cell proliferation. Its expression in breast cancer is correlated with adverse prognostic factors. It can also predict response to specific chemotherapeutic agents. Breast cancers in older women are usually less aggressive in biologic behavior. The role of topisomerase IIa in this group of women has not been widely studied.
Design: The files of the Department of Pathology, Singapore General Hospital, were searched for invasive breast carcinomas diagnosed in women aged 70 years and above, between January 1991 and December 2005. Histologic materials were reviewed, representative areas of cancers were circled for tissue microarray (TMA) construction. Sections were cut from TMA blocks and subjected to immunohistochemistry with an antibody to topoisomerase IIa using a standard streptavidin biotin method. Proportion of tumor cells stained, staining intensity, and conventional pathologic parameters were evaluated. An intensity-percentage score of >50 defined positive staining. Statistical correlation utilised SPSS for Windows, with a p value of <0.05 indicating significance.
Results: A total of 311 invasive breast carcinoma TMAs showed 92 (29.6%) cases with positive topoisomerase IIa expression. The women were aged 70 to 94 years with a median age of 73 years. Tumor size ranged from 0.1 to 14.5cm (median 3.2cm). There were 240 (77.2%) infiltrative ductal carcinomas, with the rest being mixed ductal and other subtypes. Histologic grade revealed 41 (13.2%) grade 1, 105 (33.8%) grade 2 and 128 (41.2%) cancers. Mean number of positive axillary lymph nodes was 2.8. Statistical analysis disclosed a significant association of topoisomerase IIa expression with tumor size and coexistence of ductal carcinoma in situ (DCIS), p=0.014 and 0.001 respectively. There was no correlation with histologic subtype, grade, lymphovascular invasion, or nodal status.
Conclusions: The lack of correlation of topoisomerase IIa with other pathologic parameters is unusual, and may corroborate the less aggressive nature of invasive breast cancer in this older age group. It also supports a non-chemotherapeutic approach in treatment of these cancers. The association with presence of DCIS is not readily explained, but may be related to tumor progression and persistence of the preinvasive phase of the invasive carcinoma.

215 Combined Evaluation of Pre-Operative Imaging and DCIS Grade on Core Biopsy Indicate Sentinel Lymph Node Evaluation Is Unnecessary for Most Cases of Ductal Carcinoma In Situ

AD Tatsas, JF Simpson, CR Herman, ME Sanders. Vanderbilt University, Nashville, TN.

Background: Image-guided needle biopsy is the preferred method for diagnosing breast lesions. Some examples of ductal carcinoma in situ (DCIS) diagnosed on core biopsy may be associated with invasive mammary carcinoma (IMC) in the excision specimen. Predicting such cases at the time of core biopsy would allow a one step procedure, including sentinel lymph node (SLN) biopsy.

Design: Consecutive breast core biopsy diagnoses were reviewed between 2/04 and 8/06. Cases were included for study if the following criteria were met: available pre-biopsy imaging studies, available needle biopsy and definitive excision slides, and no prior or concurrent diagnosis of IMC. The imaging studies were categorized as calcifications, asymetric density, or mass, along with the size of the lesion. Pathologic features of the core were assessed, including type and grade of DCIS, extent of core involvement, and presence of determinant calcifications and necrosis. Subsequent excisional biopsy specimens, including any SNL were also reviewed. Pathologic and radiographic features were correlated with risk of IMC and involvement of SLN at excision.

Results: 88 cases of DCIS diagnosed on needle biopsy met study inclusion criteria. All grades of DCIS were represented (HG = 32, IG = 42, LG = 14). Subsequent excisions contained IMC in 16 cases (18%); 12 No special type (NST) and 4 Special type carcinomas (tubular, lobular, medullary, and mucinous). Eleven cases had no residual lesion and the remainder contained DCIS. IMC averaged 0.78 cm (range 0.1 cm-1.8 cm). In 94% of cases which contained IMC on excision (15/16), the imaging studies showed a mass or asymetric density with or without calcifications. The single DCIS associated with microcalcifications only and with IMC on excision, was >4 cm on imaging and the only case with SNL involvement (single 3 mm focus). IMC present in excision specimens from patients with LG DCIS (3) were all low grade (tubular, mucinous, NST) whereas 17 of 18 IG/HG IMC were present in re-excision specimens from patients with IG/HG DCIS. In a single case, a pure lobular carcinoma was present in the subsequent excision of a HG DCIS.

Conclusions: SLN sampling is not necessary after a core biopsy diagnosis of DCIS unless imaging studies show a mass, or the area of micocalcification is extensive (>4 cm). Combined assessment of histopathology and imaging should allow appropriate surgical management of DCIS.

216 Comparison of a New Mouse Monoclonal Anti-HER2/neu Antibody to a Rabbit Polyclonal Antibody

DA Turbin, K Jensen, S Leung, SE McKinney, DG Huntsman, CB Gilks, RB West. Vancouver Coastal Research Institute, British Columbia Cancer Agency and University of British Columbia, Vancouver, BC, Canada; Stanford University Medical Center, Stanford, CA.

Background: The presence of HER2/neu gene amplification has been shown to predict responsiveness to trastuzumab and adriamycin. Immunohistochemical (IHC) staining for HER2 protein is used routinely in the pathology laboratories, and correlates with gene amplification, but there is no standardized methodology for IHC.

Design: We studied a widely used rabbit polyclonal anti-HER2 antibody (Ab A0485, DAKO Corp, Carpinteria, CA) and a new rabbit monoclonal antibody (clone CB11, Ventana Medical Systems Inc., Tucson, AZ) on a tissue microarray (TMA) containing 807 cases of breast carcinoma with HER2 FISH data. The patients were referred to the British Columbia Cancer Agency in the period of 1986–92. IHC stained slides were scored independently by two teams of pathologists (KJ and RW; DAT and CBG). Agreement with gene amplification data, as determined by FISH, and assessment of interobserver variability was done using SPSS 14.0 and R 2.3.1 statistical software. A permutation test with 1,000 permutations was utilized to compare differences in kappa statistics.

Results: There was a good agreement between IHC scores and FISH data, for both antibodies and both sets of observers, when the IHC scores were binarized as 0–1+ for negative and 3+ for positive cases. Better agreement with FISH was achieved using clone CB11 (kappa 0.715 and 0.752 for the two teams of observers), compared to Ab A0485 (kappa 0.667 and 0.689 for the two teams observers). The difference in kappa statistics for CB11 vs polyclonal Ab A0485 was significant for one team of observers ($p = 0.023$) but not for the other team ($p = 0.381$). Interobserver variation between the two teams for scoring the CB11 stained slides was less than for the A0485 stained slides (kappa 0.694 and 0.641, respectively). There were fewer equivocal (2+) IHC results with CB11 staining (31/807 and 28/807 equivocal cases for the two teams), compared to the A0485 stained slides (66/807 and 67/807 equivocal cases for the two teams).

Conclusions: The mouse monoclonal anti-HER2 antibody tested gave better correlation with FISH, less interobserver variation, and fewer IHC equivocal (2+) results than the rabbit polyclonal antibody.

217 Mitotic Index Evaluated with Phosphohistone H3 as Only Predictor of Outcome on Surgical Excision in Challenging Fibroepithelial Lesions

MC Uzquiano, E Resetkova, E Arribas, A Sahin. MD Anderson, Houston, TX.

Background: Classification of fibroepithelial lesions (FEL) on core biopsy (CB) can be very challenging due to the overlapping features between fibroadenoma (FA) and phyllodes tumors (PT). It is important to pre-operatively differentiate between these tumors, because PT requires surgical excision and clear margins. Our objective was to retrospectively evaluate histologically borderline FEL diagnosed on CB that had follow-up surgical excisions in our institution and set out to determine blindly if any clinical, radiologic and/or histologic parameters would distinguish between FA or PT on CB.

Design: 58 unclassified FEL on CB were retrieved from our surgical pathology files between 01/1994 and 06/2006. Clinical parameters, including age, size of lesion, and multiple parameters on imaging studies were documented. H&E stained paraffin-embedded sections of CB were evaluated for histologic pattern, stromal cellularity, myxoid changes, periductal stromal enhancement, degree of epithelial and stromal atypia, and mitotic indices. Immunohistochemistry was performed in selected cases of paraffin-embedded sections of CB using a monoclonal antibody against phosphohistone H3 (PHH3), which is directed only against cells in mitosis.

Results: In all patients clinical and/or radiologic findings were suspicious enough to indicate a CB procedure. Upon excision, the final histologic diagnoses were benign FA in 42 (72%) cases and PT in 16 (28%) cases. No statistical differences in any of the evaluated clinical and radiologic parameters were noted between FA and PT on CB. Of the histological parameters, only mitotic index was significantly increased in PT (0.88 ± 1.63 mitoses/case) compared to no mitosis seen in FA ($p<0.018$). PHH3 immunostaining improved detection rate of mitoses in PT to 3.5 ± 5.2 mitoses/case and demonstrated only rare mitoses in 3 out of 21 cases of FA (0.3 ± 0.78 per case). Those 3 lesions were small (≤ 2 cm) and radiologically benign. Moreover, PHH3 had also identified mitoses in 5 PT cases, which were not seen by H&E stain.

Conclusions: Increased number of stromal mitoses demonstrated by H&E or by immunohistochemistry for PHH3 was the only significant predictor of tumor type on excision. PHH3 is a useful adjunct to facilitate pre-operative evaluation of clinically and radiologically suspicious and histologically borderline FEL.

218 Lobulitis in Non Neoplastic Breast Tissue from Breast Cancer Patients Is Associated with Phenotypes That Are Commonly Seen in Hereditary Breast Cancer

S Vanderwerf, L Varghese, C Sweeney, C Blair, E Gulbahce. U of MN, Minneapolis; U of UT, Salt Lake City.

Background: Lymphocytic mastitis (lobulitis, ductitis, perivasculitis)is described in diabetes,autoimmune diseases and is reported in association with insitu & invasive breast cancer (BrCa). Recently, lobulitis (LL) was found to be more common in women undergoing prophylactic mastectomy for a hereditary high risk of BrCa.

Design: 334 invasive BrCa cases diagnosed 1991-96, with non-cancerous breast sections available were reviewed. Information on patients (age, menopausal status,follow-up), and tumor (type,grade,size,stage, ER/PR, and when available HER2/neu) were recorded. One section with the largest number of lobules was selected for review. Lobulitis (LL) was defined as >50 mononuclear cells (lymphocytes and plasma cells)/lobule. Mononuclear infiltrate and involved lobules were semiquantitated. Cases with LL in the areas of insitu/invasive tumor, previous biopsy/surgery site, and mononuclear infiltrates in lobules with luteal phase changes were excluded.

Results: 26/334 (8%) patients with BrCa had LL in non-neoplastic breast. Mononuclear infiltrate was mild (>50-<100) in 11, marked (>100) in 15. ≤1/4 lobules were involved in 17; >1/4-<3/4 in 8; and >3/4 in 1 case. Average age in LL:42.5, non LL:48.6. The distribution of BrCa stage, grade and HER2/neu status was similar for patients with and without LL. In a multivariable model, the presence of lobulitis was significantly associated with younger age, ER-PR- status, and certain histologies (Table).During a mean follow-up of 85 months, 0/26 LL and 57/308 non LL died of BrCa, indicating a reduced risk in LL group (p=0.02).When adjusted for other variables (stage, ER/PR,type of BrCa) no difference was seen in survival between two groups.

	Lobulitis	Non Lobulitis	p*
ER-/PR-	14/26 (53.8 %)	61/308 (19.8 %)	p=0.002
Medullary type	2/26 (7.7 %)	2/308 (0.6 %)	p=0.01
Lobular type	3/26 (11.5 %)	17/308 (5.5 %)	p=0.05

* p value from a multivariable regression model including the variables and age

Conclusions: In invasive BrCa younger age, ER-PR- status, medullary & lobular types were each independently associated with higher likelihood of presence of LL. All but the latter phenotype are known to be associated with hereditary BrCa. Prospective studies are needed to determine the significance of LL associated with BrCa and its value as a marker for hereditary high risk of BrCa.

219 Chromosome 17 Polysomy Is Common in Breast Cancer Metastases and Does Not Correlate with HER2/neu Amplification or HER2/neu 2+ Staining by Immunohistochemistry

L Varghese, S Vanderwerf, C Sweeney, C Blair, C Forster, E Gulbahce. U of MN, Minneapolis; U of UT, Salt Lake City.

Background: One cause of "false positive" immunohistochemical (IHC) testing for HER2/neu (IHC+, FISH- i.e. non amplified) is thought to be chromosome 17 (Chr17) polysomy. Some IHC 2+ or even 3+ over expressed/FISH negative cases were found to have higher Chr17 copy numbers compared to IHC 1+, indicating that gene dosage may have an impact on over expression of HER/2neu in FISH negative cases. Some studies reported Chr17 polysomy to be associated with poor prognosis and poor histologic prognostic markers.

Design: 37 patients with metastatic breast carcinoma to the axilla were identified. 1.0 mm tissue microarrays (TMA) were constructed from metastatic lymph nodes by using an average of 3.7 cores (range 1-12) from each case. HER2/neu FISH and IHC were performed on TMA blocks. Information about the patients (age, menopausal status, follow up), and tumors (type, grade, size, stage, ER/PR, and HER2neu) were recorded. Chr17 polysomy was defined as ≥2 copy/cell.

Results: 34/37(92%) cases showed concordance between IHC and FISH results on TMA. 2/37 were IHC-, FISH+; 1/37 was IHC+ FISH-(primary tumor IHC on this case was negative, indicating variable staining in core samples). 16/37(43.2%) cases showed Chr17 polysomy. Chr17 copy number correlated only modestly with HER2/neu copy number (r=0.30,p=0.08). There was no correlation between IHC2+ status or HER2/neu amplification and Chr17 polysomy (table). In a multivariable model there was no association between Chr17 polysomy and poor histologic prognostic markers (ER-PR-, grade 3, tumor size), stage, histologic type of cancer, age or HER2/neu status by FISH or IHC (+ vs- and - vs 2+ vs 3+). 5 year survival was worse for Chr17 polysomy cases but did not reach statistical significance.

		HER2/neu FISH		HER2/neu IHC		
	Average HER2 Copy No	Amplified (Average Amplification)	Non Amplified	0-1+	2+	3+
Chr17 Polysomy (n:16)	4.9	6 (3.5)	10	10	1	5
Nonpolysomy Chr17 (n:21)	3.5	7 (5.2)	14	15	1	5

Conclusions: In breast carcinoma metastases, Chr17 polysomy does not correlate with HER2/neu amplification or IHC2+ status, and does not show association with poor prognostic markers contrary to what has been reported in primary tumors in the breast. Larger number of cases need to be studied to determine the significance of Chr17 polysomy in metastatic setting.

220 A Validation Study of a Molecular Assay for the Detection of Metastases in Sentinel Lymph Nodes of Breast Carcinoma Patients

G Viale, P Dell'Orto, G Mazzarol, MO Biasi, V Stufano. European Institute of Oncology – University of Milan, Milan, Italy.

Background: An extensive histopathological examination is currently considered the gold-standard for assessing sentinel lymph node (SLN) status of breast carcinoma patients. Alternative options, based on the detection of tumor mRNA markers have been exploited to minimize labor and improve reproducibility.

Design: This prospective research study is aimed at comparing the results of a rapid molecular assay (GeneSearch™ BLN Assay) that uses RT-PCR to determine sentinel lymph node (SLN) status in breast cancer patients via quantitative analysis of mammaglobin and cytokeratin 19 mRNA with very extensive frozen section histopathologic examination. Frozen SLNs from 293 patients were completely and serially cryo-sectioned at 50 micrometers and examined histologically, while half of the intervening tissue was subjected to the molecular assay.

Results: Overall, metastases were detected histologically in 72 (24.6%) of the 293 SLNs, including 20 micrometastases. Isolated tumor cells only were identified in 10 additional SLNs, and were considered clinically negative for this study. The overall agreement of the BLN assay was 90.8%, with 77.8% sensitivity, 95.0% specificity, 83.5% positive predictive value and 92.9% negative predictive value (NPV). The assay correctly identified 50/51 macrometastases and 5/20 micrometastases based on the 50 micrometers cutting intervals used at our institution. The performance of the assay was then evaluated by simulating the histopathologic results at increasingly larger cutting intervals up to the 2mm intervals that the assay cutoffs were designed around. The sensitivity of the assay rose with increased cutting intervals, and was 96.3% (99.1% NPV) when compared with histological findings at 2 mm intervals. The histological examination of sections cut at 2 mm intervals revealed 54 of the 71 metastases detected with 50 micrometers cutting intervals, whereas the BLN assay detected 55 of the 71. The BLN Assay specificity increased with increased histological sampling.

Conclusions: This study shows that the performance of the molecular assay exceeds current published performance of standard intraoperative tests and is strictly dependent on the extent and completeness of histopathologic examination of SLNs. More extensive sectioning will detect the smaller and more infrequent metastases. The performance of the BLN Assay compares favorably to the commonly used 2 mm interval histological sectioning, aimed at detecting larger micrometastases and true metastases, per the original assay design.

221 Array Comparative Genomic Hybridization (aCGH) and Immunophenotype Analysis of a Series of 88 Ductal Carcinomas In Situ of the Breast

A Vincent-Salomon, N Gruel, C Lucchesi, V Raynal, G Pierron, B Sigal-Zafrani, P Freneaux, M Lae, RJ Salmon, A Fourquet, X Sastre-Garau, O Delattre. Institut Curie, Paris, France.

Background: To analyse the immunophenotype and the genotype of a series of high grade (HG) and non high grade (NHG) ductal carcinomas in situ of the breast.

Design: The aCGH profile, TP53 sequence and immunophenotype (ER, PR, HER2, TP53, KRT5/6, KRT8/18 and EGFR) were determined in a series of 49 HG (including 23 microinvasive cases (MI)) and 39 NHG (including 12 MI cases) DCIS.

Results: Twelve recurrent (in at least 2 tumors) amplicons, located on chromosome 1, 8, 11, 17 and 20, were identified in 51 different tumors (19/51, 37% NHG; 32/51, 63% HG). The HER2 amplicon was the most recurrent (27/88 cases), in HG (22/27; 82%), ER/PR negatives (23/27; 85%) DCIS. Interestingly, 15/27 HER2 amplified cases presented rare other alterations on aCGH profile. CCND1 was amplified in 5/88 cases (3 NHG; 2HG and 4 non MI; 1 MI). The 8q24 MYC region was gained in 12/88 cases (4 NHG; 8 HG and 8 non MI and 4 MI). A TP53 mutation was present in 10/88 cases (2 NHG, 8 HG and 4 non MI, 6 MI cases). Thirty-four regions of gains on chromosome 1, 5p, 2p, 6p, 7p, 8, 11q, 12q, 14q, 16p, 17, 19p and 22 and nineteen regions of losses on chromosome 2p, 11q, 16q, 22, 20q were observed with a recurrence rate above 10% of the 88 cases. ER positive DCIS were characterized by gains of 1q, 8, 16p, 20q, CCND1 amplification and 16q losses and a higher number of gains and losses than ER negative HER2 amplified DCIS. 5/ 88 cases (6%) demonstrated a basal-like immunophenotype not associated with a recurrent aCGH profile.

Conclusions: Our results show that (1) DCIS present a low number of recurrent alterations (2) TP53 mutations, HER2, CCND1 and MYC activation occur in DCIS and are not involved in the invasion process (3) HER2 amplified DCIS are ER negative HG tumors, classified in two groups, one presenting rare other genomic alterations beside HER2 activation (4) ER positive DCIS present a higher number of gains and losses than ER negative HER2 amplified DCIS, suggesting that ER negative and ER positive DCIS follow different carcinogenesis processes (5) A group of DCIS presented a basal-like phenotype but are not, in this small series, associated with a specific genotype. Phenotypic/genotypic characterisation should improve DCIS classification and could, in a near future, be helpful to stratify patient's therapy.

222 A Newly Proposed Semi-Automated Method of Grading Invasive Lobular Carcinoma. A Unifying Concept and Correlation with Prognostic Markers and Patient Survival

X Wang, BF Kimler, MK Davis, F Fan, P Thomas, O Tawfik. Kansas University Medical Center, Kansas City, KS.

Background: Invasive breast carcinomas are currently graded according to the "Nottingham modification of the Scarff-Bloom-Richardson system" (SBR), as recommended by the World Health Organization. This semiquantitative system evaluates 3 morphologic features: tubule formation, nuclear pleomorphism, and mitosis. In grading invasive lobular carcinoma (ILC), tubule formation is non informative as all tumors score 3/3. Additionally, most ILCs fall into an intermediate grade, which raises concerns about the SBR system's accuracy. The system is also hindered by an element of subjectivity in assessing the mitotic activity . Our recently proposed semi-automated (Kansas University-KU) grading system for invasive and in-situ mammary carcinomas has demonstrated agreement between the histologic grades and all of the histologic and prognostic markers studied. In addition, the KU system possesses improved prediction of patient survival over the SBR system. The components of the KU system are nuclear grade and proliferation index (based on percent of cells expressing MIB-1). Our objective is to extend the utilization of the KU system to grade ILC.

Design: Clinical and histopathological characteristics of 58 ILC patients treated at our institution, from 1993 to 2006, were reviewed. Tumors were evaluated using the SBR and KU grading systems, which were correlated with patients' survival information, tumor size, grade, angiolymphatic invasion, lymph node status, ER, PR, Her-2, p53, p27, p21, EGFR, BCL-2 and ploidy status.

Results: Both SBR and KU systems demonstrated overall agreement when correlated with tumor size, grade, angiolymphatic invasion, lymph node status, ER, PR, Her-2, p53, p27, p21, EGFR, BCL-2 and ploidy status. All but 2 cases were graded as either I or II by both systems. The greatest difference between the two systems was observed for those tumors initially classified as SBR Grade II with 24/30 (80%) being "down-graded" to KU I and 3/10 (30%) of SBR Grade I being "up-graded" to KU II. The KU system showed significant improvement over the SBR system in separation of the overall survival curves between Grades I and II.

Conclusions: Grading ILC is clinically important as it provides valuable predictive and prognostic information. The KU grading system for ILC appears to be superior to the SBR system in predicting patient survival and decreases the element of subjectivity for assessing mitosis.

223 Tocopherol-Associated Protein (TAP) Expression in Human Breast Cancer

X Wang, S Johnykutty, P Tang, P Bourne, SY Yeh. University of Rochester, Rochester, NY.

Background: α-Tocopherol is the principle vitamin-E homologue in human plasma. The role of vitamin-E in preventing breast cancer has been widely studied. However, the mechanism is not well known. TAP was identified as a tocopherol-binding protein in 1999, and was found universally expressed in human tissue, including the mammary gland. While promoting the anti-proliferation effect of vitamin-E, TAP itself can also function as tumor suppressor-like factor.

Design: TAP mRNA was extracted from human breast cancer cell lines (MCF-7 and MDA-MB-231) and normal breast cell line (MCF10A). Real time PCR was performed to compare TAP mRNA expression levels. Fifty cases of human invasive mammary carcinoma were identified. One tissue block with both normal breast tissue and invasive carcinoma was selected from each case. Immunohistochemical stain for TAP was performed using standard method. The staining of invasive carcinoma was scored as positive or negative by comparing with the adjacent normal breast tissue in the nucleus and cytoplasm.

Results: TAP mRNA level is significantly down-regulated in human breast cancer cell lines compared with normal breast cell line ($P<0.05$). In normal human breast tissue, TAP is expressed in the nucleus and cytoplasm of epithelial cells in segmental duct, terminal duct, and acini in a scattered fashion. Stroma and the epithelial cells of nipple and lactiferous duct are negative. More interestingly, TAP expression has the same distribution pattern as ER/PR in normal breast tissue. It appears that the same epithelial cell will either simultaneously express or not express ER/PR and TAP. In invasive carcinoma, 22 of 50 cases are TAP positive (43%), 28 cases are negative (57%). TAP expression is in association with tumor grade ($P<0.01$).

TAP expression and tumor grade

Tumor grade	TAP (+)	TAP (-)	Total
3	2	16	18
2	15	9	24
1	5	3	8
Total	22	28	50

There is no association with age, tumor size and clinical stage identified. There is no differential association with ER/PR expression identified at this time.

Conclusions: 1) TAP mRNA and TAP protein are significantly down regulated in human breast cancer cell compared with normal breast cell, indicating its tumor suppressor-like function in breast carcinogenesis. 2) TAP appears expressed in the same epithelial cells in normal duct/lobule units as ER/PR. Nevertheless more than half of ER/PR positive carcinomas are TAP negative. It further suggests the tumor suppressor role of TAP in ER/PR mediated carcinogenesis.

224 Mammaglobin, a Novel Diagnostic Marker for Metastatic Breast Carcinoma

Z Wang, B Spaulding, G Nielsen, A Sienko, Y Liang, H Li, J Ro, J Zhai. Weill College of Medicine Cornell University, The Methodist Hospital, Houston, TX; Dako North America Inc, Carpenteria, CA; Kingmed Center, Guangzhou, Guangdong, China.

Background: Breast carcinoma is known for its wide morphologic spectrum and unpredictable clinical behavior. In case of a metastatic carcinoma, with the right demographic information, breast should be considered as one of the major primaries. The current ancillary tests, however, are suboptimal in terms of sensitivity and specificity. We evaluated the diagnostic utility of mammaglobin, a member of secretoglobin family, in distiguishing breast versus non-breast carcinoma.

Design: A total of 314 formalin-fixed paraffin-embedded tissue sections were immunostained using a mouse monoclonal antibody raised against human mammglobin (clone 304-1A5; Dako, Carpinteria, CA; 1:100). These sections consisted of 41 breast primary invasive ductal carcinoma paired with 41 metastases to ipsilateral axillary lymph nodes; 160 non-breast carcinomas (59 endometrium; 35 colon; 26 lung; 15 prostate; 6 kidney; 2 stomach; 4 liver; 4 ovary; 4 pancreas; 2 thyroid; 3 undifferentiated); 36 other tumors (4 astrocytoma; 4 carcinoid tumor; 4 Ewings sarcoma; 4 Hodgkin lymphoma; 4 leiomyoma; 4 MFH; 4 melanoma; 4 mesothelioma; 4 rhabdosarcoma); and 36 specimens of normal human tissue. Immunostaining intensity was graded as 1+ (weak), 2+ (moderate) and 3+ (strong); the staining proportion was recorded as percentage of tumor area. Staining with 2+ or above and10% or more was defined as being positive.

Results: A total of 31 of 41 (76%) primary breast carcinoma were positive for mammaglobin with cytoplasmic staining pattern. Lymph node metastases paralleled with their primaries both in signal intensity and extent. The staining profile and histological grade was not correlated, however, because the majority (85%) of this cohort was moderate to high grade carcinoma. In comparison, 6 of 59 (10%) endometrial carcinoma were positive for mammaglobin; tumors of other organs and normal tissues were uniformly negative.

Conclusions: The high sensitivity and specificity highlight the value of mammaglobin as a novel diagnostic marker for metastatic breast carcinoma. An endometrial origin should be considered as the major differential diagnosis for the mammaglobin positive metastatic carcinoma, which requires a careful clinicopathologic correlation.

225 Breast Cancer Subtypes and Clinical Characteristis of Women 30 Years or Younger

YH Wen, DF Roses, DM Axelrod, AA Guth, RL Shapiro, JF Cangiarella, P Zappile, N Ziguridis, F Darvishian, B Singh. New York University School of Medicine, New York, NY.

Background: Breast cancer in adolescence and young adults is rare. The clinico-pathological characteristics of breast carcinoma in women younger than 30 years are not well described.

Design: The institutional pathology database was queried for breast cancer patients younger than 30 years of age, for the years 1991-2006. We constructed a tissue microarray and evaluated ER, PR, HER2, EGGR, CK5/6 by immunohistochemistry.

Results: Seventy-five patients were identified with very early onset breast cancer. One patient was 17 years old, 5 were between 20-24, and 69 were between 25-30 years old. Data on race was available for 34 patients (65% Caucasian, 18% Black, 12% Asian, 6% Hispanic). Most (91%) presented with a palpable mass. Family history was documented in 34 patients, and was positive in 15. Of the 75 cases, the diagnosis of adenocarcinoma, NOS was made by fine needle aspiration in 12. The remaining 63 patients had histologic material for review. Fifty-five had invasive ductal carcinoma, 1 had invasive lobular carcinoma, 3 had medullary carcinoma, and 4 were DCIS. Of the 55 invasive ductal cancers, 33 (60%) were poorly differentiated, 18 (33%) were moderately differentiated, and 4 (7%) were well differentiated. AJCC staging was assessed in 51 patients (4 Stage 0, 19 Stage I, 11 Stage IIA, 6 Stage IIB, 7 Stage IIIA, and 4 Stage IIIC). Metastasis to the axilla was noted in 25 patients. Six patients recurred, seven patients developed carcinoma in the contralateral breast, and seven patients developed distant metastases to the liver (2), the brain (2), bones (2), and the spinal cord (1) during follow-up. Immunohistochemical surrogates of the genetic classification were applied to classify tumors (Table 1).

Conclusions: Breast carcinoma in women younger than 30 years usually presents as a mass and radiologic diagnosis is uncommon. The incidence in blacks is relatively high, given the racial distribution of our surgical population. The cancers are often poorly differentiated with a poor prognosis. There is a very high incidence of basal-like subtype in very young women (34%).

Immunophenotypes of 38 patients

Breast Cancer Subtypes	# of cases	%
Luminal A (ER+/PR+ HER2-)	14	37
Lumina B (ER+/PR+/ HER2+)	2	5
HER2+ ER- PR-	6	16
Basal like (ER- PR- HER2-, EGFR and/or CK5/6 +)	13	34
Unclassified (ER- PR- HER2-, EGGR- CK5/6 -)	3	8
Total	38	100

226 Impact of Complete Removal of Breast Carcinoma by Aggressive Biopsy Techniques

YH Wen, DF Roses, DM Axelrod, AA Guth, RL Shapiro, J Cangiarella, N Ziguridis, F Darvishian, C Mercado, B Singh. New York University, New York, NY.

Background: Radiographic screening has markedly increased detection of early breast carcinoma measuring < 1cm. The advent of vacuum assisted biopsy techniques yields more tissue and has improved efficacy of core biopsy diagnosis. The purpose of this study was to evaluate the characteristics of breast carcinoma entirely removed with a core biopsy.

Design: The institutional pathology database was queried from 2003-2006 for cases of breast carcinoma diagnosed at core biopsy with no residual carcinoma identified on surgical excision. We reviewed the mammographic and pathological characteristics of these cases.

Results: We identified 21 patients in whom breast carcinoma was diagnosed by core biopsy and subsequent surgical excision (2 – mastectomy; 19 – segmental excision) revealed no residual carcinoma. All patients presented with mammographically detected clusters of calcifications and patient age ranged from 40 to 82 years (mean, 54 years). Two patients also had nonpalpable masses. Sixteen cases had 11-gauge vacuum-assisted breast biopsy with an average of 11 cores (5-20) and 5 had 14/16 gauge core biopsies. The mammographic size ranged from 4 mm to 12 mm (mean-6.3 mm). Nineteen lesions were classified as Breast Imaging Reporting and Data System (BI-RADS) 4;1 lesion was classified as BI-RADS category 3, and 1 lesion was classified as BI-RADS category 0. Seventeen cases had ductal carcinoma in situ (DCIS), 3 cases had invasive ductal carcinoma and 1 case had colloid carcinoma. Two invasive carcinomas were poorly differentiated and one was moderately differentiated. Seven cases of DCIS were high grade; 6 were intermediate and low grade each. Invasive carcinoma was seen in 1-2 cores per case with an average size of 5 mm (1-8 mm), while the average radiographic size was 8mm (5-12 mm). The average number of cores involved by DCIS was 2 (1 to 8 cores).

Conclusions: In our experience majority of mammographically detected breast carcinoma which are entirely removed at biopsy undergo 11-gauge vacuum assisted biopsy with an average of 11 cores removed. In these non-palpable mammographically detected carcinomas there maybe a discrepancy between the radiographic and pathological size, which may underestimate the stage of the carcinoma. Aggressive core biopsy techniques for diagnosis of clinically occult lesions should be discouraged.

227 Intraoperative Examination of Sentinel Lymph Node in Breast Cancer: A Comparative Study of Imprint Cytology and Frozen Section

DR Woods, R Johnson, S Lear, AW Martin, S Sahoo. University of Louisville, Louisville, KY.

Background: Intraoperative examination of the sentinel lymph node (SLN) permits a completion axillary dissection to be performed during the same procedure if metastatic tumor is found. However, the types of method used and its sensitivity in detecting metastases varies from center to center.

Design: We evaluated the sensitivity of intraoperative frozen section (FS) and imprint cytology (touch preparation (TP)) in 405 SLNs from 153 breast cancer patients performed at a single institution. Postoperative evaluation of SLN consisted of 3 to 5 serial sections of H&E stained slides cut 50 to 100 micron apart. Cytokeratin (CKAE1/3) stains were not performed routinely unless suspicious tumor cells were seen on H&E sections. CKAE1/3 stain was performed on all negative SLNs from infiltrating lobular carcinoma for this study.

Results: A total of 405 SLNs examined, 346 were true negative nodes and 56 contained metastases (54 macrometastases and 2 micrometastases) on permanent sections. There were 3 false positive cases and 6 false negative cases of FS diagnosis (2 had micrometastasis on permanent, 3 had tumor on the non-frozen portion of the lymph node, one was missed on frozen) (sensitivity 89% and specificity 99%). TPs were performed in 278 SLNs, of which 30 were true positive, 244 true negative, 2 false positive (both cases had isolated tumor cells (ITCs)), and 2 were false negative (sensitivity 94% and specificity 99%). All 29 negative SLNs of infiltrating lobular carcinoma cases were negative for CKAE1/3 stain except one, in which case ITCs were identified.

Conclusions: Both FS and TP are equally better at detecting macrometastases than micrometastases. TP is a rapid and simple technique, which can be reliably used for intraoperative evaluation of SLN for breast cancer. However, TP can be positive on cases with isolated tumor cells or micrometastases that may unnecessarily lead to complete axillary dissection. Therefore, in cases where the TP contains only a few tumor cells or groups, the findings should be confirmed with FS.

228 Heterogeneity of Breast Cancer Metastases: A Rapid Autopsy Study Using Matched Primary-Metastases Tissue Microarrays (TMAs)

JM Wu, M Halushka, D Molavi, J Fetting, NE Davidson, AM De Marzo, MJ Fackler, S Sukumar, P Argani. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Heterogeneity of biomarker expression among a patient's primary breast carcinoma (PBC) and their metastatic breast carcinomas (MBCs), as well as among different MBCs from different sites in the same patient, has not been well studied.

Design: We performed eight rapid autopsies (post-mortem intervals, 1-4 hours) on patients who died of MBC. Paraffin tissue blocks from the patients' archived PBC and multiple different MBCs were used to construct single patient TMAs. Eight TMA slides containing PBCs and in total 515 spots derived from 108 MBC sites were immunohistochemically labeled for the following: estrogen receptor (ER), progesterone receptor (PR), Her-2/*neu*, E-cadherin, Fascin, EGFR, C-Met, Cox-2, and Mesothelin. Methylation of the *RASSF1a, HIN1, Cyclin D2, Twist,* and *RAR*β gene promoters was assessed quantitatively on dissected PBC and MBC samples.

Results: Extensive heterogeneity was observed between PBC and their paired MBC, as well as among multiple MBC from the same patient. The patterns observed are summarized into three categories: 1. Markers Downregulated Uniformly in All Metastases of a Case: ER, PR. Three cases were ER-PR- and two cases were ER+PR+ in the PBC and all MBC. However, one ER+PR+ PBC was ER-PR- in all its MBC, one ER+PR- PBC was ER-PR- in all its MBC, and one ER+PR+ PBC was ER+PR- in all its MBC. 2. Markers Consistently Expressed between Primary and Metastasis. Fascin, implicated in the lung metastasis gene expression signature of PBC (Nature 2005;436: 518-524), was overexpressed in the PBC and all MBC in 1 of 8 cases. Interestingly, this was the only case with bulky lung metastasis. Promoter hypermethylation of *RASS F1A*, *RAR*β*, Cyclin D2, Twist,* and *RAR*β was very similar in the PBC and all MBC in all 7 evaluable cases. 3. Markers Variably Expressed among Metastases. E-cadherin

was variably downregulated in the MBC of one case; the E-cadherin positive invasive ductal PBC gave rise to both E-cadherin positive ductal MBC and E-cadherin negative MBC with lobular morphology. Variable overexpression in MBC compared to the PBC was observed for Cox-2 (5 cases), EGFR (4 cases), C-met (2 cases), and Mesothelin (1 case). No case strongly overexpressed Her-2/neu, but 3 cases showed variable expression ranging from negative to weakly positive (2+) in different MBC.
Conclusions: Therapeutic targets identified in the PBC or even some MBC may not reflect targets present in all metastatic sites.

229 Expression of SmgGDS in Mammary Carcinomas and Its Function in Cancer Cell Migration

C Wynveen, HY Zhi, L Gruman, JK Kuhnmuench, R Thill, CL Williams, HY Yang, SK Zhu, CG Becker, R Li. Medical College of Wisconsin, Milwaukee, WI.
Background: SmgGDS, a guanine nucleotide exchange factor, activates small GTPases that promote malignancy in different human carcinomas. Surprisingly, the expression and function of SmgGDS in human cancers has not been reported. In this study, we evaluated the expression of SmgGDS in normal breast (NB) tissues, infiltrating ductal carcinoma (IDC), infiltrating lobular carcinoma (ILC), ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS) and metastatic mammary carcinomas (Mets) using immunohistochemistry. In addition, we performed functional studies of SmgGDS using siRNA in different breast cancer cell lines.
Design: Sections from 83 primary carcinomas containing invasive carcinoma and benign breast tissue with or without in situ carcinoma, 20 reduction mammoplasties and 21 Mets were selected. In total, there were 124 cases including 40 IDCs, 43 ILCs, 37 DCIS, 32 LCIS, 21 Mets and 20 NB. Immunohistochemistry was performed using a monoclonal anti-SmgGDS antibody. In the functional studies, breast cell lines were transfected with SmgGDS siRNA to silence SmgGDS expression. The MTT assay was used to measure cell proliferation and Wound-Healing and Colloidal Gold assays were used to measure cell migration.
Results: The SmgGDS expression is listed in the table below.

Overexpression of SmgGDS in Mammary Carcinomas

IDC	DCIS	ILC	LCIS	Met	NB
36/40 (90.0%)	33/37 (89.2%)	25/43 (58.1%)	21/32 (65.6%)	21/21 (100%)	1/20 (5.0%)

SmgGDS was either weakly expressed or undetectable in benign breast epithelium. SmgGDS was overexpressed in the majority of IDC/DCIS. Interestingly, SmgGDS was overexpressed in all Mets, including cases in which SmgGDS was not overexpressed in the primary tumor. Silencing SmgGDS expression slightly inhibited cell proliferation in two cell lines. Cell migration, however, was significantly diminished.
Conclusions: 1) SmgGDS is overexpressed in mammary carcinomas, especially in ductal carcinomas, indicating the potential role of SmgGDS as a diagnostic marker in mammary carcinomas. 2) The increased SmgGDS expression in both invasive and in situ carcinomas suggests its role in early breast carcinogenesis. 3) The strong association of SmgGDS overexpression with metastasis, combined with the finding that SmgGDS enhances breast cancer cell migration, suggests that SmgGDS may play a role in tumor metastasis and thus may have clinical utility as a marker of metastasis and as a possible target in the treatment of advanced breast cancers.

230 Core Biopsy of the Breast with Atypical Ductal Hyperplasia: Implications for Clinical Management

K Yao, J Richter, E Doren, J Sinacore, PB Rajan. Loyola University Medical Center, Maywood, IL.
Background: The diagnosis of atypical ductal hyperplasia (ADH) at core biopsy (CB) is currently regarded as an indication for surgical excision. At present, there are no specific histopathologic, clinical or radiological indicators that allow us to identify those who will have more advanced lesion on excision. This study aims (1) to assess whether the cyto-archtectural features and extent of ADH are associated with an increased probability of detecting more significant lesion on excision and (2) to attempt to use these features to identify cases not requiring surgical excision.
Design: Slides from CB cases reported as ADH between 1999 and 2005 were reviewed as well as slides from subsequent excision biopsies. Degree of epithelial cell atypia on CB was graded as mild, moderate and marked (Black and Chabon 1969, Page and Rogers 1992), and the architectural pattern was grouped as solid, cribriform, micropapillary and mixed types. Extent of ADH on CB was assessed by determining the number of large ducts and/or terminal duct lobular units (TDLU) affected, with involvement of one large duct or one TDLU as representing a single focus, involvement of one duct and one TDLU as two foci, and so on (Page 2001). The Logistic Regression test was used for statistical analysis.
Results: The age of the patients ranged from 41 to 82 years. Of the 45 cases that were suitable for the study, 14 cancers were found (8 ductal carcinoma in-situ and 6 invasive carcinomas) which represented 31%. The remaining 31 cases showed either ADH or benign histology on excision comprising 69%. The degree of epithelial cell atypia ($p< 0.003$, adjusted odds ratio 35.43) and the number of foci of involvement by ADH in CB ($p< 0.058$, adjusted odds ratio 20.89) were found to be significant in the detection of advanced lesion on open biopsy. There was no association between the architectural types of ADH on CB and detection of significant lesion on subsequent excision ($p> 0.05$).
Conclusions: The degree of epithelial cell atypia and the extent of ADH in CB were found to be significant in the detection of carcinoma on open biopsy. Based on this study, we recommend that women with a CB diagnosis of mild atypia in less than 2 foci may be encouraged to follow a regular clinical and mammographic surveillance.

231 Clinicopathological Characteristics of the Triad of Tubular Carcinoma, Columnar Cell Lesions and Lobular Carcinoma In Situ

GQ Young, SM Brandt, SA Hoda. New York-Presbyterian Hospital/Weill Cornell Medical Center, New York, NY.
Background: The histological triad of tubular carcinoma (TC), columnar cell lesions (CCL) and lobular carcinoma in situ (LCIS) has been recognized, but has yet to be fully characterized.
Design: Clinicopathological material from all tubular carcinomas diagnosed at excision during a 5-year period (2001-2006) was reviewed. The diagnosis of tubular carcinoma was confirmed, and relevant data were analyzed.
Results: 86 cases of TC were studied.

Clinicopathological Characteristics of 86 Tubular Carcinomas

		Coexistent					
	n (mean age,years)	CCL*	LCIS	DCIS	Multifocal TC	Another Invasive Carcinoma**	Positive Lymph Node
All cases (mean size of TC = 0.9 cm)	86 (60)	86 (100%)	46 (53%)	35 (41%)	7 (8%)	13 (15%)	9 (10%)
Cases with triad*** (mean size of TC = 1.0 cm)	46 (60)	46 (100%)	46 (100%)	17 (37%)	5 (11%)	7 (15%)	6 (13%)
Non-triad cases**** (mean size of TC = 0.8 cm)	40 (60)	40 (100%)	0	18 (45%)	2 (5%)	6 (15%)	3 (8%)

n = number of cases. * including columnar cell change, columnar cell hyperplasia and atypical columnar cell hyperplasia. ** synchronous higher grade ductal or lobular carcinoma. *** TC+CCL+LCIS. **** TC+CCL-LCIS.
Conclusions: Tubular carcinoma is associated with a columnar cell lesion in all cases and with LCIS in 53% of cases. Tubular carcinoma with LCIS is slightly more likely to be multifocal, have another synchronous higher-grade invasive carcinoma and show nodal positivity.

232 The Molecular Classification System Is Useful for Determining the Likelihood of Complete Pathologic Response to Neoadjuvant Chemotherapy in Locally Advance Breast Cancer

M Young, NS Goldstein. William Beaumont Hospital, Royal Oak, MI.
Background: The Sorlie et al.-based immunohistochemistry molecular classification system of Abd El-Rehim, Ellis, et al. categorizes invasive breast carcinomas according to their pertinent driving biomarkers. We evaluated whether response to neoadjuvant chemotherapy correlated with the molecular classification groups in this system.
Design: 68 patients with locally advanced breast cancer treated with neoadjuvant chemotherapy were identified from our files. A immunohistochemistry molecular classification group was determined for each carcinoma giving careful attention to use the same Abs (clones and vendors) and scoring system of Abd El-Rehim, Ellis, et al. Molecular classification groups were luminal-A, luminal-B, Her2-variant (low-level amplified) (Her2-V), Her2-classic (high-level amplified) (Her2-C), and basal-phenotype. Complete pathologic response was defined as no invasive breast or metastatic-axillary carcinoma. The mean and median patient ages at diagnosis were 51.3 and 49.3 years, respectively (range, 38.2 – 83.3). Chemotherapy regimes included anthracycline based (10.5% of patients), anthracycline with taxane (70.0%) and Herceptin (19.3%). The mean and median number of resection specimen tissue blocks submitted per case were 37.9 and 35.0, respectively (range, 13 – 83).
Results: 28 of the 68 (41.2%) carcinomas had a complete pathologic response, including 2/15 (13.3%) luminal-A, 4/16 (25.0%) luminal-B, 10/12 (83.3%) Her2-classic, 0/4 Her2-variant, and 12/21 (57.1%) basal group neoplasms. The complete pathologic response rate among Her2-classic and basal group neoplasms was 66% (22/33 neoplasms), compared to 17.1% (6/35 neoplasms) in the non-Her2-classic/ basal combined group ($p < 0.001$). 11 carcinomas were initially diagnosed as invasive lobular carcinomas of which 4 were luminal-A, 4 luminal-B, 2 Her2-C, 1 basal). On review, only 3 of these 11 cases remained a lobular carcinoma, of which all were luminal-A and none had a complete pathologic response. Four of the 8 carcinomas reclassified as ductal-type had a complete pathologic response.
Conclusions: The molecular classification system is useful for guiding clinicians regarding which carcinomas are most likely (her2-classic/ basal phenotype, 66%) to undergo complete pathologic response from neoadjuvant therapy. All of the morphologically classic lobular carcinomas were luminal-A neoplasms, which may explain the low rate of complete pathologic response identified by other authors.

233 Comparison of the Sorlie-Based Immunohistochemistry Molecular Classification and Oncotype-Dx™ Recurrence Score: High Recurrence Score Neoplasms Are Comprised of Several Molecular Group Tumor Types

M Young, NS Goldstein. William Beaumont Hospital, Royal Oak, MI.
Background: The OncotypeDX™ assay (Genomics Health Inc) (OncotypeDX) commercial assay and the Sorlie et al.-based immunohistochemistry molecular classification are recently developed breast carcinoma classification systems. We evaluated the overlap and correlation between the two classifications systems.
Design: 135 invasive carcinomas with OncotypeDX assay were retrieved from our files. 29 (21.5%), 66 (48.9%), 40 (29.6%) were low, intermediate, high recurrence risk groups. A immunohistochemistry molecular classification group was determined for each carcinoma using the same Abs (clones & vendors) and classification system of Abd El-Rehim, Ellis, et al. Molecular classification groups were luminal-A, luminal-B, Her2-variant (Her2-V) (low-level amplified), Her2-classic (Her2-C) (high-level amplified), and basal-phenotype. ER+ was defined as >=4 fmol/mg cytosolic protein, to which the 1D5 ER Ab (Dako) conc. & incubation time were set so that Allred score was >2 (2180 case validation set). Image analysis was applied to 1179 of these cases to set cut-points and subsequently used clinically.

Results: The distribution of carcinomas by molecular classification and OncDX recurrence score groups is listed in Table 1. For the 26 Luminal-A carcinomas, the mean/ median OncotypeDX recurrence scores were 13.4/ 13.5 respectively (range, 8 – 20). None of the Her2-V, Her2-C, or basal molecular groups had low recurrence risk OncotypeDX scores. Of the 40 high OncotypeDX recurrence score cases, 0 were luminal-A, 9 (22.5%) were Luminal-B, 8 (20.0%) were Her2-V, 14 (35.0%) were Her2-C, and 9 (22.5%) were basal phenotype. All of the Her2-C and basal phenotype cases had very-low level positive ER values.

Conclusions: The Sorlie-based IHC molecular classification groups and OncotypeDX recurrence score overlapped to a substantial degree. Luminal A group carcinomas were highly correlated with low OncDX recurrence score. Although ER+, the high recurrence risk OncDX score group was comprised of a mixture of neoplasms including Her2-classic and basal phenotype neoplasms.

OncotypeDX Recur. Score vs Molec. Class. Group

	OncDX Recurrence Score			
Molec. Class. Group	**Low**	**Intermediate**	**High**	**Total Cases**
Luminal-A	24 (92.3%)	2 (7.7%)	0	26 (100%)
Luminal-B	5 (7.4%)	53 (79.1%)	9 (13.4%)	67 (100%)
Her-Variant	0	7 (46.7%)	8 (53.3%)	15 (100%)
Her2-Classic	0	2 (12.5%)	14 (87.5%)	16 (100%)
Basal	0	2 (18.1%)	9 (81.8%)	11 (100%)

234 Correlations of Chromosome 17 Number with Her2/neu Gene Copy Number by FISH and Protein Expression by IHC in Breast Invasive Ductal Carcinoma

S Zhang, SS Shah. SUNY-Upstate Medical University, Syracuse, NY.

Background: Her2/neu gene amplification by fluorescent in-situ hybridization (FISH) and protein expression by immunohistochemistry (IHC) have been used for prognosis and guiding treatment of invasive ductal carcinoma of the breast with Trastuzumab. FISH assay is considered as a gold standard in the interpretation of cases with equivocal IHC results. Polysomy of chromosome 17, at which Her2/neu is located, can be identified in 20-30% of cases tested and it is unclear whether this affects the interpretation of FISH and IHC results. The aim of this study was to correlate the chromosome 17 number with Her2/neu gene copies by FISH and protein expression by IHC.

Design: A retrospective analysis of invasive ductal carcinomas diagnosed over 18 months revealed 90 cases that were evaluated for Her2/neu gene by FISH and protein expression with IHC. Clinical history, histologic slides and FISH results were evaluated. The Her2/neu/CEP17 ratio, Her2/neu copy number per nucleus and number of chromosome 17 per nucleus was calculated. The FISH data were compared with IHC results through computing correlation coefficients (CC) and statistical analysis.

Results: FISH for Her2/neu gene was performed on 90 women (Mean age 57.9 years; Range 26-92 years). The average chromosome 17 number per nuclei was 2.4 (1.5 to 3.95). There were strong associations of IHC grading with FISH Her2/neu/cep 17 ratio (CC 0.51, $p < 0.001$) and Her2/neu signal/nucleus (CC 0.51, $p < 0.001$), as expected. In addition, we found significant correlation between number of chromosome 17/nucleus and Her2/neu signal per nucleus (CC 0.44, $p < 0.001$). However, no correlation was seen between number of chromosome 17/nuclei and either IHC grading (CC 0.18, $p > 0.05$) or Her2/neu/cep17 ratio (CC 0.17, $p > 0.05$). Polysomies (≥2.7 chromosome /nuclei) were identified in 23 of 90 (25.6%) cases.

Conclusions: Polysomy occurs commonly in invasive ductal breast carcinomas. Although the chromosome 17 copy number influences the total Her2/neu signal per nucleus, it affects neither the ratio of Her2/neu/cep17 by FISH nor the protein expression by IHC.

235 Triple Approach to Core Needle Biopsy (CNBX) of Breast Masses with and without Image-Guidance: Follow-Up Analysis of 673 Patients with Benign CNBX Diagnoses

Z Zhang, M Raoufi, A Ormsby, U Raju. Henry Ford Hospital, Detroit, MI.

Background: Sampling error is an expected limitation of CNBX. Triple approach has been successful for fine needle aspiration biopsy of breast lesions. Radiologic pathologic (rad-path) correlation is required for image-guided CNBX (I-CNBX). Actively seeking the imaging and clinical information helps to identify a discordant pathology findings of a highly suspicious clinical and/or radiographic mass.

Design: 673 patients with benign CNBX diagnoses in 2003, performed for clinical and/or radiographic mass-lesions [213 non-image guided (N-CNBX) and 460 I-CNBX] were analyzed for missed cancer. The diagnoses were grouped into 1) specific (SP) for mass lesion (correlated well with a mass; eg. fibroadenoma, papilloma, cyst, nodular sclerosing adenosis, complex sclerosing lesion); and 2) nonspecific (NSP) (uncertain or discordant for mass; eg. stromal fibrosis, fibrocystic change, usual hyperplasia, mild sclerosing adenosis, fibroglandular or fatty breast tissue NOS).

Results: All cases were followed for 6 to 1328 days (mean 826.23). There were 13 missed cancers (1 in SP group, 12 in NSP-group); 4 were I-CNBX and 9 were N-CNBX. By imaging, 5 were birad 5 (highly suspicious), 6 were birad 4 (suspicious); 2 had no imaging. The interval from the benign CNBX to diagnosis of malignancy was 10 days to 9 months. In NSP group (n =220), 109 had I-CNBX and 111 had N-CNBX. Missed cancer rate was highest for N-CNBX if there was also a mass-lesion detected by imaging (7/45); there were no cancer when imaging was negative (0/46). Discordance was recognized and cancer diagnosis was quickly established in 10 patients including all cases of I-CNBX (average 25.88 days ± 14.81 SD), however cancer diagnosis was delayed by > 4 months in 3 N-CNBX cases.

Conclusions: 1). Missed cancer rate is highest for non-image guided CNBX with non-specific benign diagnosis if there is also a mammographic and/or ultrasound mass. 2).The existing rad-path correlation protocols for image-guided CNBX effectively prevent delay in cancer diagnosis. 3). By using triple approach, pathologists and surgeons can recognize possible discordance between the pathologic findings and clinical/ radiologic mass lesion, potentially leading to prompt re-biopsy and definitive diagnosis.

	CNBX	I-CNBX	N-CNBX	Nonsp-group, I-CNBX	Nonsp-group, N-CNBX	Nonsp-group; pos imaging mass, N-CNBX	Nonsp-group; neg imaging, N-CNBX
Total	673	460	213	109	111	45	46
Missed cancer (n)	13	4	9	3	9	7	0
Missed cancer (%)	1.9%	0.8%	4.2%	2.7%	8.1%	15.6%	0

236 Prognostic Significance of EGFR and Phosphorylated EGFR Expression in Invasive Breast Cancer

C Zoubouli, C Magkou, I Giannopoulou, K Karali, S Markaki, E Mylona, L Nakopoulou. Attikon Hospital, Athens, Greece; Medical School, University of Athens, Athens, Greece.

Background: Epidermal growth factor receptor (EGFR) is involved in regulating cell growth in breast carcinomas. Its activated form (pEGFR) is correlated with poor prognosis in lung cancer, whereas it has not yet fully investigated in breast cancer. The aim of this study was to investigate the expression of EGFR, pEGFR and their correlation with overall and disease free survival, clinicopathological parameters (nuclear grade, histological grade, hormonal status, tumor size and stage), the antiapoptotic Bcl-2 and biological markers of invasion and metastasis (uPAR, MMP-9, VEGFR-1/Flt-1).

Design: A three-step immunohistochemical method (ABC/HPR) was applied on paraffin-embedded sections from 156 patients with invasive breast carcinoma to detect the expression of the proteins EGFR, pEGFR, ER, PR, Bcl-2, uPAR, MMP-9 and VEGFR-1/Flt-1. The results were statistically processed using chi-square test. Overall and disease-free survival distribution curves were assessed by Kaplan-Meier test and log-rank statistics followed by Cox's proportional hazards regression model.

Results: EGFR and pEGFR proteins were immunodetected in the membrane of the malignant cells (11.9% and 35.7% respectively), and less commonly in the normal epithelium and in situ component, where they existed. EGFR expression was positively correlated with nuclear grade ($p=0.004$) and negatively correlated with hormonal receptors ER and PR ($p<0.001$ and $p=0.048$, respectively) and bcl-2 ($p=0.008$). pEGFR was positively related to VEGFR-1/Flt-1, uPAR and MMP-9 ($p=0.001$, $p=0.049$ and $p=0.026$, respectively). Univariate analysis showed that only pEGFR-positive cases had poor overall survival ($p=0.017$), a finding that is further supported by multivariate analysis ($p=0.002$).

Conclusions: These data provide clinical evidence that pEGFR expression seems to be an important independent prognostic factor and it is related to angiogenesis and invasiveness. EGFR immunoexpression is related to nuclear grade and hormonal status, and therefore to cellular differentiation.

Cardiovascular

237 Surgical Pathology of Bicuspid Aortic Valve: A 10 Years Survey

C Basso, S Rizzo, A Abudurheman, G Gerosa, G Thiene. University of Padua Medical School, Padua, Italy.

Background: To report the surgical pathology experience on bicuspid aortic valves (BAV) collected in a ten years time interval at a pathology institution serving a single Cardiac Surgery Department.

Design: The medical charts and BAV specimens from patients undergoing aortic valve replacement in the time interval 1994-2003 were retrospectively reviewed. A detailed gross and histologic examination of excised valves was carried out.

Results: Among 1480 pts who underwent aortic valve replacement, 166 pts had BAV (11%). They were 121 M and 45 F, age ranging 1-79 yrs, mean 55±16 (vs 66±12 of the tricuspid aortic valve (TAV) pts, $p<0.0001$). The mean age at operation was 58±15 in BAV pts with aortic stenosis vs 46±15 in BAV pts with pure aortic incompetence ($p=0.0001$). The M/F ratio was 2.7 in BAV vs 1.28 in TAV ($p<0.0001$). 70% of pts presented had stenosis due to dystrophic calcification, 26% had pure incompetence and 4% aortic dissection. Isolated valve replacement was done in 72%, valve replacement plus ascending aorta substitution in 21% and aortic valve replacement plus aortoplasty in 7%. The mean prosthetic diameter was of 24±1.8mm in BAV pts vs 22 ±1.9mm in TAV pts ($p<0.0001$). A raphe was present in 60% of pts. No difference as far as presence of raphe and cusp symmetry was found in stenotic vs incompetent BAV (65% vs 66%, and 66% vs 38%, respectively, p= NS). Pure aortic regurgitation occurred mostly in the setting of aortic root pathology (85%). Overall, infective endocarditis occurred in 6% of BAV pts vs 2% of TAV pts ($p=0.001$). 84 pts were operated due to aortic dissection and a BAV was identified in 8%.

Conclusions: BAV is the underlying substrate of aortic valve dysfunction in 11% of pts requiring surgery, with a strong male predominance. The most common fate of congenitally BAV is stenosis due to dystrophic calcification (70%). Pure aortic incompetence is mostly due to concomitant aortic root pathology. BAV is a not so rare substrate for infective endocarditis and dissection.