performed a more thorough evaluation of the prevalence and degree of TFE3 immunoreactivity in GCT .

Design: A tissue microarray (TMA) was assembled from formalin-fixed and paraffin-embedded tissue blocks of 37 cases of GCT and one case of ASPS, and contained four 0.6 mm cores of tumor tissue from each case. Of the patients with GCT, 19 were male, and 18 were female with an average age of 46.3 yr (range 9 – 71 yr). The primary sites were skin (20), tongue (4), soft tissue (6), vulva (2), breast (2), esophagus (2), and palate (1). TFE3 immunostains were performed and scored as described in the above study.

Results: Nuclear immunoreactivity for TFE3 was observed in 34 of 37 (92%) GCT, and in the positive control ASPS case. Of the 34 TFE3 positive GCTs, 11 (32%) showed strong (3+) and 23 (68%) showed moderate (2+) nuclear labeling. The majority of TFE3 positive cases (30/34, 88%) showed diffuse reactivity with 75 – 100% nuclei positive while the remainder show reactivity in 30 – 75% of nuclei. TFE3 nuclear labeling was not seen in control normal tissues (skin, liver, adipose tissue, skeletal muscle, breast) or other neoplasms (schwannoma, fibroadenoma, phylloides tumor, rhabdomyosarcoma, melanoma) included in the TMA.

Conclusions: The prominent and frequent nuclear TFE3 immunoreactivity of GCTs precludes its use in discriminating GCTs from ASPS and is so far unique among tumors without known TFE3 translocations. Its basis may warrant further investigation.

Breast

94 ZO-1 and Occludin - Novel Markers of Lobular Carcinoma of Breast

B Agarwal, S Mehrotra, A Morimiya, S Badve. Indiana University, Indianapolis, IN.

Background: Expression of E-cadherin, an adhesion molecule, has been used to distinguish lobular carcinoma from ductal carcinoma of breast. During the development of cell-cell junctions cadherin associate with a number of proteins including tight junction proteins Zonula Occludens- 1 (ZO-1) and Occludin. These proteins normally localize to the apical aspect of the breast epithelial cells. Expression of ZO-1 is associated with gland formation in breast cancer and has been reported in low grade invasive ductal carcinomas. As lobular carcinoma does not exhibit gland formation, we hypothesized that the distribution of these polarity related proteins might be altered in lobular carcinoma and could be used as markers of this disease. In this study we examine the expression of ZO-1 and Occludin in lobular cancer of breast and differentiating it from ductal carcinoma of breast.

Design: Archival tissue specimens from 20 patients having lobular carcinoma of which 12 had a lobular carcinoma in-situ breast cancers were analyzed for the expression of Occludin (Zymed, pre-diluted) and ZO-1 (Zymed, pre-diluted) by immunohistochemistry.

Results: Both ZO-1 and Occludin showed an apical zonal expression in normal luminal epithelial cells and this was seen in all cases and served as an internal control. ZO-1 expression was also noted in endothelial cells.

ZO-1 and Occludin expression was completely lost in invasive component in 16/20 cases (80%) of invasive lobular carcinoma. Loss of normal apical distribution was seen in the other four. This was in the form of diffuse cytoplasmic expression in 3 cases and focally membranous expression in one case. ZO-1 and Occludin expression was completely lost in 11/12 (92%) cases of lobular carcinoma in-situ and showed altered distribution in the form of diffuse cytoplasmic staining in one case.

Conclusions: Loss of cellular polarity as indicated by expression of tight junction proteins ZO-1 and Occludin is an early event in lobular carcinoma. It is seen in both invasive and in-situ lesions. Loss and/or abnormal localization of adhesion proteins could give rise to the diffuse pattern of growth characteristic of lobular cancer of breast. Since alteration in normal distribution was seen in all the lesions examined, it appears that ZO-1 and Occludin could be good markers for the diagnosis of lobular carcinoma. Further studies are ongoing to assess the utility of these markers in distingiushing lobular carcinomas from ductal lesions.

95 Lymphangiogenesis Does Not Occur in Breast Cancer

B Agarwal, S Mehrotra, A Morimiya, R Saxena, S Badve. Indiana University, Indianapolis, IN.

Background: Lymph node metastasis is one of the best indicators of prognosis in breast cancer. Although the importance of angiogenesis in hematogenous spread of breast cancer is well established, little is known about the invasion and generation of lymphatic vessels. This is due to lack of markers that specifically identify lymphatic vessels. VEGF-C and LYVE-1 have been previously used for this purpose but they lack specificity and often do not distinguish lymphatic endothelium from blood endothelial cells. Recently, D2-40 has been described as a novel specific marker for identification of lymphatic vessels. In this study we used dual immunohistochemistry (with D2-40 and PCNA) to identify and study lymphangiogenesis in breast cancer.

Design: Double immunohistochemistry was performed on paraffin sections from 25 patients having breast cancer for D2-40 (Signet Lab, dil 1: 40) antibody and polyclonal PCNA (dilution 1: 400). A lymphatic vessel density count was performed by noting the expression of D2-40 in the lymphatics. The lymphatic endothelial proliferation was studied by studying the expression of PCNA in the tumour cells as an internal control.

Results: Lymphatic vessels as identified by D2-40 expression were seen in the peritumoral area. However, lymphatics were not identifed within invasive tumors, except in areas adjacent to pre-existing ducts and lobules. The lymphatic endothelial cells did not show any expression of PCNA indicating minimal or no proliferative activity. This was in contrast to strong expression in adjacent tumor cells, which served as internal control.

Conclusions: Whether breast tumor cells co-opt and invade existing lymph vessels or induce proliferation of new lymphatic vessels is not known.Our findings suggest that lymphangiogenesis does not occur in breast cancer and it is likely that breast cancer cells utilise pre-existing lymphatics for metastasis. In contrast to angiogenesis, lymphangiogenesis does not seem to play a major role in the metastatic invasion of breast tumors.

96 Core Biopsy Specimens with and without Calcifications: Should They Be Submitted Separately?

TM Alasio, K Skinner, A Simsir, J Cangiarella. New York University, New York, NY.

Background: Mammotome core biopsy of mammary microcalcification (MC) ia a commonly used diagnostic method. Radiologists perform core specimen radiographs to document the presence of MC. For the surgical pathologist identification of MC is critical to ensure appropriate sampling of the radiographic finding. Cores "with calcification" and cores "without calcification" are submitted in individual containers and the final pathology report contains separate diagnoses for the cores with and without MC. We reviewed pathology reports from cases of MC to investigate whether this separation was necessary.

Design: Computerized search yielded 119 11-gauge mammotome core biopsies from 110 female patients (age range 33 to 81 yrs) with MC. Cores were radiographed and separated according to the presence or absence of MC and placed in formalin containers marked "with calcification" and "without calcification". Cores were paraffin-embedded, sectioned with three levels and stained with hematoxylin and eosin. The diagnoses from the individual containers were compared and differences were analyzed.

Results: In 93 cases (78%) there was no difference in diagnosis between cores marked as "with calcification" and "cores without calcification". The majority of these cases (66%) yielded a benign diagnosis. The remaining 33% were DCIS (17), invasive carcinoma (13) or atypical ductal hyperplasia (1). In 26 cases (22%), the diagnoses were different. In 21 cores with MC and in 5 cores without MC, differences between the two diagnoses would have led to changes in patient management ($p \leq 0.001$, chi-square test). Results are summarized in Table 1.

Conclusions: In the majority of mammotome biopsies for MC (78%), separation of cores by specimen radiography did not contribute to a difference in pathologic diagnosis, regardless of the presence or absence of MC. A difference in diagnosis that would lead to a change in patient management was noted in 22%. In 10 cases, separation of cores allowed a definitive diagnosis of malignancy in cores containing MC in comparison to those without MC.

Comparison of diagnoses of mammotome core biopsy specimens with and without microcalcification

		Cores without calcification			
Cores with calcification	Non diagnostic	Benign	Atypical Hyperplasia	DCIS	Invasive carcinoma
Non diagnostic					
Benign	3	62	3*		
Atypical Hyperplasia		4	1*		
DCIS		9	3**	17	2
Invasive Carcinoma		1		1	13

*Atypical ductal hyperplasia in all cases, **2 cases atypical ductal hyperplasia, 1 case atypical lobular hyperplasia

97 Prognostic Significance of Combined Use of Histologic Grade and Ki-67 in Breast Cancer

C Alenda, FI Aranda, R Durán, FM Peiró, G Peiró, J Seguí, E Adrover. Hospital General Universitario de Alicante, Spain.

Background: Grading systems based in Patey-Scarff and Bloom-Richardson, later modified by Elston has been applied and validated repetitively in large series of breast cancer cases. The proliferative capacity of neoplasms is one of the most crucial variables for tumor grading. More recently, immunohistochemical quantification of Ki67 has been applied in routine study of proliferative activity in breast cancer. However, the significance of combined use of histologic grade (HG) and Ki67 is not well established.

Design: Formalin-fixed, paraffin-embedded tissues from 244 invasive breast cancers were stained with Ki67 (monoclonal MIB-1, 1:50; Dakocytomation). Cases with ≥20% of stained tumor cells were considerate as high-Ki67. In all cases, Nottingham combined histologic grade (HG) was applied. A combination based on HG and Ki67 was applied as following: low grade (HG I and low-Ki67), intermediate grade (HG I-II with high-Ki67 or HG III with low-Ki67), and high grade (HG II-III with high-Ki67). Median follow-up was 59 months (range 4-102). The univariate relationship between variables and overall survival (OS) was analyzed by the Kaplan-Meier method, and the differences were assessed by the log-rank test. All statistical manipulations were performed using the SPSS for Windows system.

Results: Tumors with HG III contained high level of Ki67 ($p<0.000$). OS rate of cases with low-Ki67 was 89% and with high-Ki67 75% ($p=0.0021$). OS rate in HG I was 92 %, in HG II 84%, and in HG III 77% ($p=0.0192$). OS rate in low-grade tumors was 94%, in intermediate-grade 85%, and in high-grade 74% ($p=0.0024$). OS rate in lymph node negative patients (n=170) was as follows: low-Ki67 97% vs. high-Ki67 82% ($p=0.0005$); HG I 97 %, HG II 93% and HG III 84% ($p=0.0237$); low-grade 100%, intermediate-grade 92% and high-grade 80% ($p=0.0012$).

Conclusions: Histological grade and immunohistochemical detection of Ki67 represent two parameters that provide prognostic information, independently of the stage of the disease. Combination of both allows a better selection of risk groups in patients with breast carcinoma and defines a group with excellent prognosis.

98 Locally Advanced Breast Cancer. A Study of Predictive Factors of Pathological Response to Treatment with Primary Chemotherapy

FJ Andreu, A Sáez, M Sentís, JC Martín, M Rey. UDIAT-Centre Diagnòstic - Corporació Parc Taulí, Sabadell, Barcelona, Spain.

Background: Miller & Payne's histolocial classification of pathological response (PR) to primary chemotherapy treatment (PCT) is the only one that correlates the different grades of PR with survival rate. The predictive value of different biomolecular factors in this group of patients has not yet been established. Objectives: 1) The evaluation of PR of patients with LABC treated with PCT. 2) The study of possible association of several biomolecular factors with response to treatment.

Design: Prospective study of 60 patients with LABC (2002-2003). Standard PCT and surgery after PCT. PR assessment using Miller & Payne grading system: five grades of local response - G1: no reduction in overall cellularity; G2: < 30% reduction ; G3: 30-90%; G4: >90% and G5: total response (DCIS may be present) - and four grades of lymph node (LN) response (A: negative LN; B: positive LN; C: partial LN response and D: total LN response). IHC study of hormonal receptors (ER/PR), proliferative index (Ki67), expression of TP53, EGFR and HER-2/neu and gene amplification (HER-2 and Topoisomerase IIα) by CISH in diagnostic large needle core biopsy. Bivariant statistical analysis of results.

Results: 1) Mean age 52.3 (range 29-79). 2) Invasive ductal carcinoma (IDC) 48 cases (80%) and invasive lobular carcinoma (ILC) 12 cases (20%). 3) Lumpectomy: 12 cases (20%) and mastectomy: 48 (80%). 4) Local pathological response: G1-G2: 11 (18%); G3-G4: 38 (63%) and G5: 11 (18%). 5) Correlation of local and LN response: G5: 91% of LN (-) (64% total LN response and 27% negative); G3-G4: 84% LN (+)(66% partial response and 18% no response); G1-G2: 73% (+) LN (46% partial response and 27% no response). 6) For ILC, G3-4 local response: 92%; G2: 8% and LN response C: 50%; B: 42% and A: 8%. 7) Bivariant analysis: G5 associated to CDI type in 100% (p=.07); negative ER and PR in 73% (p=.003 and .03), and high proliferative index (Ki67 >25%) in 100% (p= .009). No other evidence of statistically significant association.

Conclusions: 1) Identification of 18% of patients with total response. 2) Local total response associated to IDC and significantly to high Ki67 index and negativity for ER/PR. 3) Significant association between local and lymph node pathological response. 4) High incidence of ILC, no cases of local or lymph node total response.

99 Claudin-7 Has Different Expression Pattern in the Breast Cancers Compared to the Colorectal Cancers

YK Bae, JH Choi, MJ Kim. Yeungnam University Medical Center, Daegu, Korea.

Background: Claudins are transmembrane proteins that seal tight junctions, and are critical for maintaining cell-to-cell adhesion in epithelial cell sheets. Claudin-7, one of the 20 identified members of the claudin protein family, is known as differentially expressed in breast cancer relative to normal breast epithelium. However, its role in cancer progression remains unexplored. The aim of this study is to determine the value of claudin-7 in breast cancer progression.

Design: Paraffin embedded, formalin fixed archival tissue of ductal carcinoma in situ (DCIS) (n=42) and invasive ductal carcinoma (IDC) (n=142) of the breast was retrieved from the pathology files. Claudin-7 expression was evaluated immunohistochemically using Claudin-7 antibody (Zymed) and tissue microarrays. We included 422 cases of colorectal carcinoma (CRC) for the study and compared their expression patterns to those of the breast cancers. Immunohistochemical staining was scored on a semiquantitative scale of 0-3+.

Results: Claudin-7 was expressed in normal mammary epithelium and colonic mucosa (2-3+). In contrast, there was complete loss of claudin-7 expression (0+) in gastric mucosa (control), regions of intestinal metaplasia showed 2-3+ staining. Claudin-7 was expressed in 69% (29/42), 27.5% (39/142), and 96.9% (409/422) of DCIS, IDC, and CRC, respectively. Loss of claudin-7 expression (0-1+) was correlated with invasiveness (p<0.001) in breast cancers and with nuclear grade in both DCIS (p=0.03) and IDC (p=0.03), occurring predominantly in high-grade tumors. Claudin-7 expression was not correlated with tumor size, lymph node metastasis, and stage in IDC.

Conclusions: Our data suggest an important role of Claudin-7 in breast cancer progression (invasion) and prognosis. In CRC, Claudin-7 does not seem to be involved in dissemination of cancer cells because most CRCs showed strong immunostaining for Claudin-7.

100 Flat Epithelial Atypia in Breast Needle Core Biopsies: A Correlative Followup Study

H Bai, CJ Sung, Q Wu, JT Machan, MR Quddus, WD Lawrence, MM Steinhoff. Women and Infants Hospital, Brown Medical School, Providence, RI.

Background: Flat epithelial atypia (FEA) of the breast is encountered more frequently due to the increasing use of needle core biopsies (NCB) for mammographically detected abnormal microcalcifications. The clinical significance of FEA is uncertain and few follow-up studies are available in the literature. This study aims 1) to evaluate the correlation between FEA in needle core biopsies and subsequent pathologic findings in needle localization excisions (NLE); 2) to compare the significance of FEA in atypical ductal hyperplasia (ADH).

Design: 65 cases of needle core biopsies with the diagnoses of columnar cell changes (CCC), columnar cell hyperplasia (CCH), atypical CCH and ADH with subsequent needle localization excisions from the period 2000-2004 were reviewed. Columnar cell lesions in needle core biopsies were classified as CCC/CCH (4 cases), FEA (4 cases), ADH with FEA (35 cases) and ADH with no FEA (22 cases). Cases of FEA with coexisting ductal carcinoma in-situ (DCIS), lobular carcinoma in-situ (LCIS), and invasive mammary carcinoma (IMC) in needle core biopsies were excluded. Fisher's Exact test with an overall significance level of 0.05 was used to compare the subsequent needle localization excision results of ADH with FEA and ADH with no FEA in needle core biopsies.

Results:

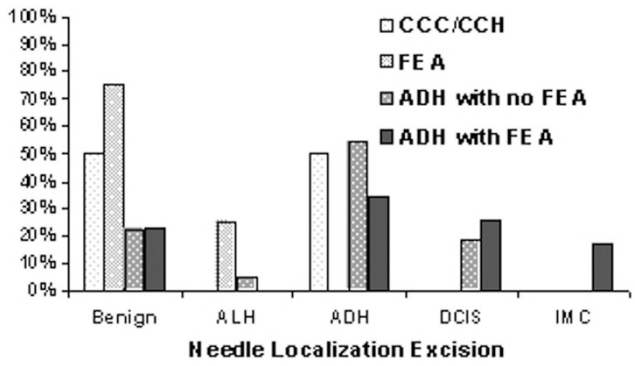


Table 1

NCB		NLE
	DCIS/IMC (%)	Benign/ALH/ADH (%)
ADH with FEA (n=35)	15 (43%)*	20 (57%)
ADH with no FEA (n=22)	4 (18%)*	18 (82%)

**p=0.048*

Conclusions: The results demonstrate that 43% of ADH with FEA in needle core biopsies have a worse lesion in the needle localization excisions either as DCIS or invasive mammary carcinomas, appearing to be significant in comparison with that of ADH with no FEA (18% with DCIS) in subsequent excisions (p <0 .05). The association of invasive mammary carcinomas (2 tubular and 4 infiltrating ductal carcinoma) further highlights the significance of ADH with FEA, although a larger sample size might be required to draw more definitive conclusions.

101 Implant-Associated Mesenchymal Tumors (IAMT) of the Breast: A Fortuitous or Causal Association?

BL Balzer, SW Weiss. Emory University, Atlanta, GA.

Background: Implant associated mesenchymal tumors of the breast are extremely rare, and there has been no systematic study of this association to determine whether evidence supports a causal or fortuitous relationship.

Design: All mesenchymal tumors associated with a breast implant were retrieved from our consultation files noting temporal relationship to graft placement, type and intactness of graft, and location of tumor. Immunohistochemistry for cytokeratin was performed in selected cases to rule out metaplastic carcinoma. Immunostains for beta catenin were performed in 3/5 cases of fibromatosis.

Results: Seven IAMT were identified exclusively in female patients (ages 28-64 yrs; median 49 yrs) who presented with a palpable mass. All tumors followed placement of either a silicone (n=6) or saline (n=1) implant which had been employed for cosmetic purposes (n=6) or following surgery for breast carcinoma (n=1). One patient had Poland syndrome, but none was known to have polyposis coli or Gardner syndrome. All IAMT arose in the immediate vicinity of a grossly intact implant but also involved the chest muscle (6) and breast parenchyma (1). IAMT included 5 typical fibromatosis which expressed beta catenin (3/3), 1 low grade fibrosarcoma, and 1 high grade undifferentiated pleomorphic sarcoma. The last was confined entirely to implant capsule and was negative for cytokeratin. In patients with silicone implants, silicone granulomas were intimately associated with the neoplasm. All patients were treated with local excision. Follow-up ranged from 2 to 4 yrs, with a mean of 3 years. One patient had 2 local recurrences of fibromatosis following implant replacement, and the other 6 patients are alive without recurrences or metastases.

Conclusions: IAMT of the breast are rare tumors which may occur with either saline or silicone implants. The majority are typical fibromatoses. There is some circumstantial evidence that IAMT may be causally related to the implant since they develop: 1) following implant placement; and 2) in the immediate vicinity of the implant or within the implant capsule. However, since the majority of IAMT are fibromatoses, the mechanism of tumorigenesis may not be a reflection of the introduction of foreign material per se, but rather a result of surgical trauma, as has been demonstrated in other forms of fibromatosis, such as those occurring in the setting of Gardner syndrome.

102 BRCA2 Associated Breast Cancer: Histopathologic and Biomarker Profiling

AL Bane, E Shi, S Tjan, T Earle, C Have, IL Andrulis, FP O'Malley. Mount Sinai Hospital, Toronto, ON, Canada; Samuel Lunenfeld Research Institute/Mount Sinai Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.

Background: There is increasing evidence that hereditary cancer syndromes resulting from germline mutations in cancer susceptibility genes exhibit organ specific cancers with distinct histologic phenotypes. Several studies have shown that hereditary breast tumors resulting from germline BRCA1 mutations are usually high grade invasive ductal (no special type) carcinomas with pushing margins, a high mitotic index and are negative for ER, PR and HER2/neu protein overexpression. Fewer studies have assessed BRCA2 associated breast cancers and a distinguishing histologic and biomarker profile has yet to be definitively identified. The objective of our study was to determine the morphologic phenotype of BRCA2 associated breast cancers and to determine their biomarker profile.

Design: 62 BRCA2 associated breast cancers were compared with 105 sporadic breast cancers, matched for age and ethnicity. All tumors were accrued through the Ontario Familial Breast Cancer Registry. Centralized pathology review was performed with the pathologist blinded to the mutational status of participants. Odds ratios were derived for each parameter of interest and the results are tabulated below. Tissue microarrays (TMAs) were constructed from the carrier and control tumors and utilized for the assessment of ER, PR and HER2/neu protein expression by immunohistochemistry and HER2/neu gene amplification by FISH.

Results: The majority of the 62 BRACA2 associated tumors were ER positive (77%) and PR positive (70%). Only 7 of 62 (11%) tumors were positive for HER2/neu overexpression and/or gene amplification. There was no statistical difference between the carrier and control population for biomarker expression.

Conclusions: BRCA2 associated breast cancers are predominantly high-grade invasive ductal (no special type) carcinomas. They often exhibit pushing margins, similar to BRCA1 associated tumors, and frequently have co-existent DCIS. Despite their high histologic grade these tumors are usually ER and PR positive, while HER2/neu protein overexpression is infrequent.

Variable	Carrier	%	Control	%	Odds Ratio
No. of Invasive Cancers	62		105		
Primary Morphology	Ductal NST 60	95	Ductal NST	87	1.00
Grade I/III	3	5	21	20	1.00
Grade II/III	18	29	37	35	3.21
Grade III/III	41	66	47	35	5.70
Pushing margins present	22	35	18	17	2.60
DCIS Present	50	79	69	66	1.70

103 Molecular Clonality Relationships of Distant Metastases Following Ipsilateral Breast Failure (IBFs) in Patients Treated with Breast Conserving Therapy: Some Distant Metastases Derive from IBFs and Evidence That Metastases Metastasize

N Baniseed, NS Goldstein, S Hunter, FA Vicini, LJ Kestin. William Beaumont Hospital, Royal Oak, MI.

Background: Whether IBFs can be can be the source of distant metastases (DMs) following breast conserving therapy (BCT) and directly impact survival is controversial. Additonally, the question of whether metastases can metastasize remains largely speculative. We studied the clonality relationships of invasive breast carcinomas with invasive IBFs and biopsied DMs using a PCR-LOH assay to the IBF to study these questions.

Design: Carcinoma DNA was extracted from paraffin blocks, analyzed with 20 markers to common tumor suppressor genes. LOH was defined as +/- 50% allelic loss relative to the allelic ratio of normal tissue.

Results: In two cases, the initial and IBF carcinomas were clonally related, DMs were clonally related to the IBFs but shared minimal genetic features with the preceding initial carcinoma. The IBF and DM were both clonally related to the initial carcinoma and appeared to be one progressively genetic unstable process in two cases. Both the IBF and DM were clonally related to the initial carcinoma but unrelated to each other in one case. The IBF was clonally different from the initial carcinoma and the DM was clonally related to the IBF in one case. In the two cases with two DMs in different organs that occurred 0.4 and 0.8 years apart, the second DMs had the same, additional LOHs seen in the first DM which were not present in the preceding breast carcinomas.

Conclusions: The clonality relationships between initial carcinomas, IBFs, and DMs is more complex than models suggested by prior authors. DMs can directly result from IBFs in some cases. However, not all the preceding IBFs which metastasize are clonally related to the initial carcinoma, some are new second carcinomas. Some initial carcinomas appear to have multiple subclones with different and unrelated IBF and DM potential. In the two studied cases, second generation DMs were clearly derived from preceding DMs, evidence that some metastases metastasize.

104 Pure Spindle Cell Metaplastic Tumors of the Breast: An Immunohistochemical and Morphologic Study with Follow-Up

R Barner, GL Bratthauer, FA Tavassoli. Armed Forces Institute of Pathology, Washington, DC; Yale University School of Medicine, New Haven, CT.

Background: Metaplastic tumors of the breast are a heterogeneous group of neoplasms that have been variably categorized. Recent studies have investigated the clinicopathologic characteristics, biologic behavior, and immunohistochemical properties of a unique subset of metaplastic tumors of the breast, those with low-grade spindle cell morphology. Excluding fibromatosis, nodular fasciitis, myofibroblastoma and its variants, as well as, carcinomas (ductal or squamous cell) with spindle cell metaplasia, mammary tumors with low grade spindle cell morphology have been most often described as variants of metaplastic carcinoma or potentially myoepithelial in origin. The current study describes a continued investigation of low grade spindle cell tumors to clarify their morphologic characteristics, immunohistochemical profile, local recurrence potential, and metastatic potential.

Design: Breast tumors with predominantly spindle cell morphology were selected from the AFIP files from 1984 to 2003. This study was limited to breast tumors with low grade nuclear atypia and spindle cell morphology. Thus, tumors with intermediate to high grade nuclear atypia, or squamous, ductal, or adenosquamous epithelial elements were excluded. Clinical, pathologic, and immunophenotypic features [pancytokeratin, 34BetaE12 (K903), CK 5/6, calponin, smooth muscle actin, CD 10, ER, CD 34, and p63), were reviewed, with emphasis on biologic behavior.

Results: 48 tumors were comprised entirely of low grade spindle cells. Fibromatosis-like, periductal, myxoid/edematous (nodular-fasciitis-like), and rare inflammatory growth patterns were observed. The tumors were consistently and strongly positive for p63, 34BetaE12, CK5/6, variably positive for pancytokeratin, while ER, CD 34, and calponin were negative. CD10 and SMA stained myofibroblastic stroma associated with most tumors. Follow-up showed that five tumors had local recurrence (1 to 4 years), one tumor metastasized to an axillary lymph node, and two tumors metastasized to the lungs.

Conclusions: Our findings support previous studies of low grade spindle cell tumors of the breast. The cellular immunoprofile has overlapping features of myoepithelial cells and squamous cells, suggesting a cell of origin. These tumors display unique histologic characteristics and immunoprofile, have the potential for local recurrence, rare distant metastases, and low morbidity.

105 HER-2 and Topoisomerase II-alpha Gene Amplification and Protein Overexpression in Invasive Breast Cancer: Chromogenic In Situ Hybridization and Immunohistochemical Analyses

R Bhargava, P Lal, B Chen. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Co-amplification of HER-2 and topoisomerase II-alpha (topo2a) genes has been reported in breast carcinomas. HER-2 gene amplification most often results in protein overexpression, however gene to protein correlation for topo2a is not entirely clear. We studied HER-2 and topo2a gene amplification, and protein overexpression in 113 selected invasive breast carcinomas using tissue microarrays.

Design: Gene amplification was studied using chromogenic in situ hybridization (CISH) and protein expression was studied using immunohistochemistry. The Spot-Light® HER-2, topo2a, and chromosome 17 centromeric probe kits (Zymed Laboratories Inc.) were used for CISH analysis. A ratio of gene copy number (HER-2 or topo2a):chromosome 17 copy number ≥2.0 was interpreted as positive for gene amplification. A ratio of topo2a:Chromosome 17 of <0.8 was interpreted as gene deletion. HER-2 protein overexpression was scored according to standard guidelines for HercepTest™. A tumor was interpreted as positive for topo2a protein overexpression when nuclear staining was identified in >5% of the tumor cells.

Results: One hundred and four of the 113 tumors were successfully analyzed for both HER-2 and topo2a gene amplification. Sixty-four of 104 tumors showed HER-2 amplification. Twenty-five of these 64 tumors (39%) also showed topo2a amplification. Forty tumors did not show either HER-2 or topo2a amplification. Deletion of the topo2a gene was seen in 7/64 (11%) of HER-2 amplified tumors and in 2/40 (5%) of HER-2 non-amplified tumors. Eighteen of the 25 tumors (72%) with topo2a amplification also showed topo2a protein overexpression. In contrast, only 3 of the 79 tumors (3.8%) without topo2a amplification showed topo2a protein overexpression.

Conclusions: Topo2a amplification is closely associated with HER-2 amplification, but not *vice versa*. Topo2a gene amplification results in protein overexpression in approximately three quarters of the tumors. In contrast, topo2a protein overexpression rarely occurs in the absence of gene amplification. Identification of topo2a status along with HER-2 gene status may have therapeutic and prognostic implications.

106 Epidermal Growth Factor Receptor (EGFR) Gene Amplification and Protein Expression in Breast Carcinoma: A Study of 175 Tumors

R Bhargava, P Lal, W Gerald, B Chen. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: HER family of receptor tyrosine kinase has been extensively studied in breast cancer, however systematic studies of EGFR gene amplification and protein overexpression in breast carcinoma are lacking.

Design: EGFR gene amplification was studied by chromogenic in situ hybridization (CISH) (EGFR SpoT-Light DNA probe, Zymed Laboratories Inc.) and protein expression was studied by immunohistochemistry (IHC) (anti-EGFr antibody, clone 31G7, Zymed) using tissue microarrays in 188 breast carcinomas. Tumors with >5 EGFR copies per nucleus were interpreted as positive for gene amplification. Protein overexpression was scored and analyzed similar to standard criteria used for HercepTest™. EGFR mRNA level was studied using Affymetrix U133 gene chip in some of these tumors. HER-2 gene amplification (using Vysis FISH and Zymed CISH) and protein overexpression (Dako HercepTest™) were also studied in all tumors.

Results: Of the 188 tumors studied, gene amplification and protein expression were successfully analyzed in 175 (93%) tumors. EGFR gene amplification was detected in 11/175 (6.3%) tumors, and protein overexpression was found in 12/175 (6.9%) tumors (Table 1). Eight of the 11 tumors (73%) with gene amplification also showed protein overexpression (IHC 2+ or 3+). Two of three tumors with gene amplification but no protein overexpression also failed to show mRNA overexpression. The mRNA data was not available in the other tumor. Two of these 11 tumors showed HER-2 amplification and overexpression. Of these 164 tumors negative for EGFR amplification, 4 (2.4%) tumors were positive for protein overexpression (Table 1). The gene copy number in these 4 tumors ranged from 2-5. EGFR mRNA data was not available in these 4 tumors.

Conclusions: EGFR amplification or overexpression is an infrequent event in breast carcinoma and shows no apparent relationship to HER-2 amplification. A strong EGFR protein overexpression appears to correlate with gene amplification. The EGFR mRNA level was apparently not increased in tumors showing gene amplification but no protein overexpression, although the number of cases is very small. Breast carcinomas may occasionally show EGFR protein overexpression by IHC without gene amplification, and the exact mechanism needs to be further investigated.

Correlation of EGFR gene amplification and protein expression.

EGFR-IHC	Amplified	Not Amplified	Total
0	2 (1.3%)	149	151
1+	1 (8.3%)	11	12
2+	2 (40%)	3	5
3+	6 (85.7%)	1	7
Total	11 (6.3%)	164	175

107 Pleomorphic Lobular Neoplasia (PLN): Histologic and Phenotypic Findings from Needle Core Biopsies (NCB) and Their Subsequent Surgical Excisions Are in Favour of a Pre-Invasive Entity

F Bibeau, P Michenet, M-C Chateau, C Borrelly, R Lavaill, B Masson. CRLC Val d'Aurelle, Montpellier, Herault, France; Hopital de la Source, Orleans, Loiret, France.

Background: Lobular neoplasia is considered to be a marker of cancer risk, but its appropriate management is still controversial. The increasing use of NCB, for non palpable breast lesions, has highlighted evolving entities such as PLN. Our goal is to emphasize this pleomorphic variant, whose presentation differ from the classical form of lobular neoplasia and whose recognition has clinical relevance.

Design: Eleven PLN were primarly diagnosed from ten 8 or 11 Gauge (G) Mammotome® procedures, using stereotactic mammography and one 14 G biopsy, using ultrasound guidance. All PLN, but one, were mammographically detectable, because of microcalcifications. Subsequent surgical specimens were obtained in 10 cases, during which 6 sentinel lymph node biopsies or 2 axillary dissections were performed. An immunohistochemical study for estrogen and progesteron receptors (ER, PR), c-erbB2, E-Cadherin and high molecular weight cytokeratin 34 beta E-12 (HMW-CK), was realized on NCB and excision specimens.

Results: Among the 11 PLN cases, 3 were associated with an invasive lobular carcinoma (ILC) and 4 with a microinvasive lobular carcinoma (<1 mm). Six invasive or microinvasive components were directly identified on NCB. One ILC was idenfied on the surgical resection alone. ILC and microinvasive carcinoma were always closely located to the PLN areas, both on biopsies and surgical specimens. Morphologically, PLN cases were composed of pleomorphic, dyscohesive cells with comedo-like, histiocytoid and signet ring features. Comedo-like patterns with central necrosis were associated with microcalcifications. PLN presented the following phenotype : ER 9+ /11, RP 5+/11, CerbB2 2+/11, E-cadherin 0+/11, HMW-CK 8+/11. PLN and their invasive/microinvasive counterparts shared a similar phenotype. One metastatic axillary involvement was noted.

Conclusions: These clinical, histological and phenotypic data support the evidence that PLN looks more like a preinvasive lesion than a marker of increased risk. Thus, percutaneous biopsies with PLN should be evaluated diligently for the presence of an invasive or microinvasive lobular component and require a subsequent surgical excision. Pathologists, as well as physicians involved in the management of breast disease, should be aware of this mammographically detectable entity, which has distinc histologic pattern and behaviour.

108 Evaluation of Epidermal Growth Factor Receptor (EGFR) and Estrogen Receptor (ER) in Primary Breast Cancers and Lymph Node Metastases Using Tissue Microarray

C Bicquart, K Kawase, MKP Garcia, D Rosen, WF Symmans, J Liu, CT Albarracin. Univ of Texas, M. D. Anderson Cancer Center, Houston, TX.

Background: Overexpression of EGFR has been reported in 30% to 60% of breast cancers, and has been shown to correlate with negative hormone receptor status and poorer clinical outcomes. Recently, the use of tissue microarrays in assessing receptor expression has been shown to have a significant advantage allowing simultaneous processing under identical conditions. Tissue microarrays will be useful for the validation of tumor markers from a large number of tissue specimens. The purpose of this study is to examine the expression of EGFR and ER in primary and metastatic breast cancers using tissue microarray.

Design: Primary breast tumors and their corresponding axillary lymph node metastases were obtained from 68 cases. Duplicate 1 mm cores were sampled from each primary and metastatic carcinoma tissue. Immunohistochemical staining for EGFR and ER were performed. Staining for EGFR was scored, membranous staining was considered positive and the percentage of positively staining cells were noted. Staining for ER was scored as follows: negative, <10% staining and positive, ≥10 staining. Correlation of EGFR expression, ER expression, and various patient and tumor characteristics were examined.

Results: EGFR was positive in 21% of primary tumors (12/58 cases) and 36% of lymph node metastases (21/59 cases). This shows a trend for higher EGFR expression in lymph node metastases as compared to the primary carcinoma. ER was positive in 75% of primary tumors (42/56 cases) and 69% of lymph node metastases (42/61 cases). EGFR expression in primary tumors and their corresponding lymph node metastases have a high correlation using the Spearman correlation coefficient ($p<0.00001$). A significant inverse correlation between EGFR and ER was observed. No correlation was found between EGFR and any of the tumor characteristics, such as tumor size, grade, and the number of lymph node metastasis.

Conclusions: Our results are consistent with other studies which have been performed on tissue sections, suggesting that tissue microarray is reliable to assess marker expression in primary and metastatic breast cancer. The increased EGFR expression in lymph node metastasis indicates that EGFR has a role in disease progression, which may contribute to the poor clinical outcomes associated with this marker.

109 Distribution of FISH Scores in Amplified Breast Carcinomas Derived from 9546 Consecutive Cases

KJ Bloom, D Helling, D Bouman. ChromaVision Medical Systems, Irvine, CA; US Labs, Irvine, CA.

Background: The assessment of HER-2 gene amplification is typically performed by Fluorescence in-situ hybridization (FISH). Probes are directed against the HER-2 gene and the centromeric portion of chromosome 17, to differentiate true gene amplification from chromosomal aneusomy. Little data is available on the distribution of FISH scores on a large number of breast cancers. This study looks at the distribution of the HER-2:CEP-17 ratio is 9546 consecutive breast cancers submitted for FISH analysis.

Design: 9546 consecutive breast cancers assessed for HER-2 gene amplification at US Labs were reviewed. FISH was performed using the PathVysion assay (Vysis, Downer's Grove, IL). Twenty cells were assessed and the number of HER-2 and CEP-17 signals were counted in each cell, up to twenty signals each. The HER-2:CEP-17 ratio was rounded to the closest integer category for those tumors showing a ratio greater than or equal to 2.0.

Results: 2163 tumors (22.6%) showed a HER-2:CEP-17 ratio of greater than or equal to 2.0. The distribution of the ratio of amplified tumors ranged from 2 to 15. 562 of these tumors (26%) had a ratio of less than 3. The remaining tumors showed a roughly normal distribution with a mode of 6 and a mean of 6.5. 1298 tumors (60%) had a ratio between 4 and 11 and only 14% had a ratio greater than 10. Of the 562 tumors with a ratio of less than 3, 426 (76%) had an average HER-2 count of 6 or less.

Conclusions: The distribution of HER-2:CEP-17 ratio is roughly normally distributed with a mean of 6.5 and a mode of 6. Most of the tumors with a HER-2:CEP-17 ratio between 2 and 3 do not appear to be the result of HER-2 gene amplification but rather the result of reduced CEP-17 counts. The purpose of normalizing the number of HER-2 signals to the number of chromosome 17 signals is to distinguish tumors with true gene amplification from polysomy in tumors showing increased HER-2 signals. When the average number of HER-2 signals is not increased, the determination of a HER-2:CEP-17 ratio may give the false appearance of gene amplification in some cases.

110 Remarkably High Frequency of EGFR Expression in Breast Carcinomas with Squamous Differentiation

V Bossuyt, O Fadare, M Martel, B Burtness, RL Camp, FA Tavassoli. School of Medicine, New Haven, CT.

Background: The human epidermal growth factor receptor (EGFR) is reportedly over expressed in 20-40% of breast carcinomas. EGFR over expression is associated with reduced survival and is inversely correlated with expression of ER and PR. This study assessed EGFR expression in breast carcinomas with squamous differentiation.

Design: The immunohistochemical expression of EGFR was evaluated in 30 breast carcinomas with squamous differentiation (5 pure squamous, 25 adeno-squamous) using the pharmDx assay (clone 2-18C9, DakoCytomation). Cases were considered positive if at least 10% of the cells showed 1+ positivity in the squamous component. Squamous differentiation was confirmed with immunostain for CK5-6 (clone D5/16B4, DakoCytomation) on selected cases. ER, PR and HER2 status as well as clinical information regarding treatment and outcome were correlated. A tissue microarray comprising 280 lymph node positive breast carcinomas was evaluated with the same EGFR assay.

Results: The 30 patients, ranged in age from 21 to 77 years (mean 52). The tumors measured 1.3-30 cm (mean 4.8). Sentinel or full axillary lymph node dissection was performed in 21 patients. Ten patients had positive lymph nodes. At the time of initial diagnosis 3 patients had distant metastasis. Follow-up was available for 17 patients (mean 33 months). Disease free survival at 3 years was 58% (1 death from unrelated causes, 5 deaths from disease at 1-56 months, 3 patients recurred after 3-4 years).

Table 1

	Total	ER-	ER+	PR-	PR+	HER2+	HER2-
Total	**30**	**19**	**4**	**20**	**3**	**7**	**11**
EGFR+	**26**	18	1	18	1	5	11
EGFR-	**4**	1	3	2	2	2	0

87% (26/30) of tumors were positive for EGFR. EGFR positive tumor cells (showing squamous morphology) were also found in 4/5 lymph node and 1 bone metastasis available for EGFR assay. Correlation of EGFR status with ER, PR and HER2 status is shown in Table 1. All but one of the EGFR+ tumors examined were ER and PR negative. All pure squamous carcinomas were EGFR+. Only one of the EGFR- cases showed positive immunostaining with CK5-6. No statistically significant differences in size, lymph node status and disease free survival were observed between EGFR+ and EGFR- cases. Nine tumors (3%) on the tissue microarray were EGFR+. Review of the initial diagnostic slides failed to reveal squamous features in all but one case.

Conclusions: Breast carcinomas with squamous differentiation are a morphologically and clinically distinct subgroup of breast carcinomas with a very high frequency of EGFR positivity. Breast carcinomas of this type would be ideal candidates for treatment with EGFR inhibitors.

111 BTG2 Expression in Human Breast Cancer

EF Brachtel, H Kawakubo, S Maheswaran. Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Background: BTG2 is an anti-proliferative protein that is expressed at variable degrees in normal breast epithelium during ontogenesis. BTG2 expression in the mammary glands of rats is decreased at pregnancy and lactation, but up-regulated during involution. Estrogen and progesterone, which induce proliferation in the breast, suppress BTG2 mRNA *in vitro,* and cell culture studies demonstrate that BTG2 suppresses breast cancer cell growth. However, little is known about the role of BTG2 in human breast cancer development. In this study, we examined BTG2 mRNA and protein expression in human invasive ductal carcinoma (IDC), and correlated the results with histologic grade and estrogen receptor (ER) status.

Design: 23 cases of IDC were selected from the files of Massachusetts General Hospital Pathology Department. Formalin-fixed, paraffin-embedded sections containing tumor and adjacent uninvolved glands were stained with the BTG2 antibody using standard immunoperoxidase techniques after antigen retrieval. Specificity was tested by incubation with COS cell protein lysates derived from vector- and BTG2 transfected cells. Nuclear staining was evaluated semi-quantitatively, from negative to strongly positive, and set in relation to adjacent uninvolved breast tissue, which served as internal positive control. Corresponding fresh tissue stored at -80°C from 5 cases was

used for Northern blot analysis. RNA was separated on a formaldehyde gel, transferred to membrane and probed with human BTG2 cDNA.
Results: BTG2 protein expression in the tumor was less than in normal glands in 15/23 cases (65%), 11 of which were poorly differentiated (G3/3), 3 moderately differentiated (G2/3), and 1 well differentiated (G1/3). 11 were ER+, 3 ER-. Two cases were also negative for BTG2 mRNA. 8/23 (35%) cases showed similar BTG2 staining intensity of tumor compared to normal, 2 cases were G3, 3 were G2, and 3 were G1. 7 cases were ER+, 2 ER-. One case was negative for BTG2 mRNA.
Conclusions: BTG2 mRNA and protein expression appear to be suppressed in invasive ductal carcinoma compared to uninvolved breast epithelium. Those with BTG2 downregulation were more likely high grade IDCs. Our findings support the *in vitro* observation that BTG2 suppresses breast cancer cell growth.

112 Is There a Ductal Carcinoma In Situ (DCIS) Counterpart to Invasive Basal-Like Breast Cancers?

BB Bryan, SJ Schnitt, LC Collins. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.
Background: Recent gene expression profiling studies have identified a distinct subtype of breast cancer known as basal-like carcinomas (BLC). These lesions comprise about 15% of invasive breast cancers (IBC), are often seen in women with BRCA1 mutations, and are characterized by lack of expression of ER and PR, lack of HER2 protein overexpression, and expression of one or more basal cytokeratins (CKs). These studies have further indicated that the majority of ER/PR/HER2-negative (triple negative) IBC cluster as BLC. BLC are histologically poorly-differentiated ductal carcinomas and presumably have a DCIS precursor lesion with similar cytologic and immunophenotypic features. However, the frequency and even the existence of a distinctive DCIS lesion with an immunophenotype similar to that of BLC has not been studied in detail.
Design: To address this issue we studied 56 cases of high nuclear grade DCIS (HGDCIS) to determine the frequency of the triple negative phenotype and the expression of basal CKs in these lesions. Each case was immunostained for ER, PR, HER2 and basal CKs (5/6, 14 and 17). A case was considered basal CK-positive when neoplastic cells comprising the DCIS showed cytoplasmic staining for one or more of these CKs.
Results: Among these 56 HGDCIS cases, 4 (7%) exhibited the triple negative phenotype and the remaining 52 cases showed other combinations of ER, PR, and HER2 expression (non-triple negative). Staining for one or more of the basal CKs was detected in 19 of the 56 cases (34%), and in all cases was focal. Basal CK expression was seen 3 of the 4 triple negative DCIS (75%), but in only 18 of the 52 non-triple negative DCIS (35%), although this difference did not reach statistical significance (p=0.10). Thus, overall 3 of the 56 HGDCIS cases (5%) showed a phenotype similar to that of basal-like IBC (ER/PR/HER2-negative and basal CK positive).
Conclusions: In an analysis of 56 HGDCIS, we identified 3 cases (5%) that exhibited an immunophenotype typical of that reported for basal-like invasive breast cancers (i.e., ER/PR/HER2-negative and basal CK-positive). Given that invasive breast cancers typically share immunophenotypic features with the DCIS from which they arise, our findings raise the possibility that the triple-negative, basal CK-positive DCIS lesions we identified represent the precursor lesion to basal-like invasive breast cancers. Their relative infrequency may be due to the fact that basal-like carcinomas evolve rapidly and largely obliterate the DCIS from which they arise.

113 Do the Results of Breast Biomarker Studies Differ bewteen Core Biopsy and Surgical Excison Specimens?

C Burge, S Apple. UCLA Medical Center, Los Angeles, CA.
Background: Core biopsies are commonly used in the diagnosis of breast cancer and many times are the only sample available to provide prognostic and predictive markers prior to neoadjuvant chemotherapy. Therefore, an accurate correlation between core and excisional biopsy specimens is extremely important in order to guide the proper treatment of these lesions.
Design: We retrospectively studied 87 patients with breast cancer to compare the concordance rates for type of tumor, grade, ER/PR status and Her2neu by immunohistochemistry and FISH between core biopsy and excisional biopsy specimens.
Results: The histologic type of cancer had a 100% concordance rate between core and excisional biopsy specimens. The concordance rate of modified Bloom and Richardson score between core and excisional biopsy specimens was 77% (67/87). Among the 20/87 (23%) discordant cases, 17/20 (85%) were upgraded mostly due to mitotic counts in the excisional biopsy and 3/20 (15%) were downgraded due to overall tumor nuclear pleomorphism. The concordance rate for ER was 95% and PR was 89% between core biopsy and excisional biopsy specimens. The concordance rate for Her2/neu by immunohistochemistry was 96% between core and excisional biopsy specimens. The concordance rate for Her2neu by FISH was 100% between the core and excisional biopsy specimens. The concordance rate for Her2neu by immunohistochemistry and FISH was 94%.
Conclusions: Our study shows that relatively high concordance rates can be obtained when comparing core biopsy and excisional biopsy specimens. As such, core biopsies can be used as a reliable means of providing accurate information regarding prognostic and predictive tumor markers prior to neo-adjuvant chemotherapy.

114 Encysted (Noninvasive) Papillary Carcinomas Are Associated with Pseudoinvasion More Often Than Microinvasion

BC Calhoun, RA Jensen, JF Simpson, ME Sanders, DL Page. Vanderbilt University Medical Center, Nashville, TN.
Background: Encysted noninvasive papillary carcinoma is distinguished from more common types of ductal carcinoma in situ by its circumscription in an encysted focus. Such lesions show dominant involvement by a pattern of DCIS, often have low to intermediate nuclear grade, and are adequately treated by complete local excision (Carter et al. *Cancer* 52:14, 1983). We report the frequency of finding pseudoinvasion, microinvasion (≤1.0mm), and minimal invasion (>1.0-5.0mm) in association with an encysted noninvasive papillary carcinoma.
Design: All cases with a diagnosis of encysted papillary carcinoma from the Vanderbilt Breast Pathology Consultation service from 1996 through 1999 were retrieved. For a diagnosis of micro- or minimal invasion, extension beyond the encysting fibrous tissue and histologic features of invasive mammary carcinoma were required.
Results: Of the 304 cases, 53 (17%) showed pseudoinvasion of the encysting fibrous tissue, 7 (2%) showed microinvasion, and 28 (9%) showed minimal invasion. Pseudoinvasion and microinvasion were both present in two cases. DCIS was present in ducts and micropapillomas outside the encysted focus (using criteria of Page et al. *Cancer* 78:258, 1996) in 155/304 cases (51%). Five of 7 cases with microinvasion (71%) and 18/28 cases with minimal invasion (64%) had DCIS in surrounding breast tissue. Among cases with no micro- or minimal invasion, 132/269 (49%) had DCIS in adjacent breast parenchyma.
Conclusions: Many cases of pseudoinvasion had been misinterpreted as microinvasion by some reviewers. Our data indicate that micro- and minimal invasion are less commonly associated with encysted carcinomas than pseudoinvasion. The associations in this study may be skewed by being part of a consultation service but should have the potential to guide conservative therapeutic options. If completely excised, we expect that the presence of low grade T1a tumors would not significantly alter the excellent prognosis of encysted noninvasive papillary carcinoma.

Table 1

	Microinvasion (≤1.0mm)	Minimal Invasion (T1a)	Pseudoinvasion
No. of cases	7/304 (2%)*	28/304 (9%)*	53/304 (17%)*

*two cases with pseudoinvasion and micro- or minimal invasion

Table 2

	Microinvasion (≤1.0mm)	Minimal Invasion (T1a)	No Invasion	All Cases
DCIS Outside	5/7 (71%)	18/28 (64%)	132/269 (49%)	155/304 (51%)

115 Separate Cavity Margin Samping at the Time of Initial Lumpectomy Significantly Reduces the Need for Re-Excision

D Cao, C Lin, S Woo, T Tsangaris, P Argani. The Johns Hopkins Hospital, Baltimore, MD.
Background: In breast conservation therapy, margin status predicts local recurrence and determines the need for re-excision. Many surgeons now take, at the time of lumpectomy, separate "cavity margins" (CMs) as final margins that supercede the specimen margins (SMs) . There is little data on the efficacy of this method.
Design: 126 patients [23 with ductal carcinoma in situ (DCIS) and 103 with invasive carcinoma (IC) +/-DCIS] who had an oriented lumpectomy specimen and also had 6 additional CMs (superior, inferior, medial, lateral, anterior and posterior) taken with the lumpectomy were studied. The neoplasms were evaluated for the following: size, grade, SM status (distance of tumor from margin and extent of involvement), vascular invasion, lymph node status, presence or absence of extensive intraductal component (EIC). The additional CMs were evaluated for residual carcinoma (if any) and its distance from the inked true margin, and the results correlated with the corresponding SM. Margins were considered clinically positive if tumor was within 2mm of the inked surface, since this generally triggers re-excision at our institution. Otherwise, margins were considered clinically negative.
Results: In 23 cases, all SM were clinically negative; in these cases, all CM remained negative. Among the 103 cases with at least one clinically positive SM, additional CM rendered the overall final margin status clinically negative in 62 (60%) while at least one margin remained clinically positive in 41 (40%). Presence of EIC was strongly predictive of residual carcinoma in the CMs following clinically positive SMs ($p < 0.01$), but tumor size, grade, vascular invasion and nodal status were not. At individual margin level, when carcinoma was transsected at a SM, its corresponding CM contained tumor in only 36.5% of cases, and in only 19.4% if the carcinoma was within 1 mm of the inked SM. The extent of involvement of the SM (aggregate length in mm of carcinoma within 1mm of the margin) did not predict tumor in the corresponding CM.
Conclusions: CM specimens taken from areas corresponding to positive SMs usually lack tumor, and therefore many positive SMs are likely false positives. Possible factors causing false positive SMs include seepage of ink into crevices of main specimen, manipulation of specimen for radiographs, and retraction artifact. When a SM is clinically positive, only the presence of EIC in the tumor predicts residual carcinoma in CM. Additional CM sampling diminishes the need for re-excisions.

116 The Phosphatidylinositol 3'-Kinase Signaling (PI3K) Pathway in *In-Situ* and Invasive Breast Carcinoma: A Tissue Microarray Analysis

S Chandran, N Bose, J Mirocha, S Khan, S Bose. Cedars Sinai Medical Center, Los Angeles, CA.
Background: The PI3K pathway is an important regulator of cell proliferation and survival. The pathway is known to be deregulated in many cancers including breast. Dissecting the molecular events associated with activation of this pathway in breast cancer patients *in vivo* presents an important challenge that has implications for the development and clinical testing of PI3K pathway inhibitors.

Design: A tissue microarray containing 140 cases of invasive breast carcinomas and 120 cases of pure ductal carcinoma in-situ (DCIS) was constructed. Immunohistochemical analysis using antibodies to the PTEN protein and to the phopsphorylated forms (p-) of its downstream effector proteins – AKT (also known as protein B kinase), FKHR (a member of the Forkhead family of transcription factors), mTOR (the mammalian target of rapamycin), and S6 (a ribosomal protein) – was performed on consecutive sections. Cyclin D1 (Bcl1) expression was also determined. Results were assessed using Spearman correlation, Fisher's Exact test, Student's t-test and multivariable logistic regression.
Results: Invasive carcinomas and DCIS showed loss of PTEN expression in 26% and 11.5% cases respectively and overexpression of pAKT in 38% and 33%, pFKHR in 20% and 15%, pmTOR in 24% and 32%, pS6 in 72% and 47% and Bcl1in 70% and 60% cases respectively. Difference in the expression of the various proteins, in DCIS and invasive carcinoma was significant only with PTEN and pS6, indicating that aberrant expression of these two proteins is associated with tumor progression. Additionally PTEN loss correlated significantly with pS6 overexpression . Significant correlations were also observed between pAKT, pmTOR and pS6 and between pFKHR , pmTOR and pS6 indicating that both pAKT and pFKHR are associated with overexpression of downstream proteins pmTOR and pS6.
Conclusions: We demonstrate that loss of tumor suppressor protein PTEN occurs with progression of DCIS to invasive cancer and this is associated with overexpression of pS6. Although PTEN is known to antagonize PI3K pathway activation, and is associated with activation of the main PI3K effector, AKT, we did not observe this in our cases. AKT was, however, overexpressed in more than 30% of DCIS and invasive carcinomas indicating that other mechanisms of AKT activation maybe effective early in breast carcinogenesis. We also show that activation of AKT and FKHR are associated with phosphorylation of mTOR and S6, a finding that has been previously reported in experimental models.

117 Medullary and Atypical Medullary Carcinoma of the Breast: Expression of *BRCA-1*, HLA-DR and Beta-2 Microglobulin and Lack of Evidence of Epstein-Barr Virus Infection

ED Chang, EJ Lee, CS Kang. Catholic University of Korea, Seoul, Korea.
Background: Medullary carcinoma (MC) is unique in that it is associated with a heavy lymphocytic infiltrate and a relatively good prognosis considering its ominous cytological features. Atypical medullary carcinoma (AMC) is histologically similar to MC but has a prognosis similar to that of invasive duct carcinoma. Major histocompatibility complex (MHC) molecules are important in regulating the immune response against tumors. The purpose of this study was to examine the expression of *BRCA-1*, human leukocyte antigen (HLA)-DR and beta-2 microglobulin in MC and AMC and to see if these lesions were related to Epstein-Barr Virus (EBV) infection.
Design: Expression of *BRCA-1*, HLA-DR and beta-2 microglobulin was examined by immunohistochemical methods in twelve cases of MC and tens cases of AMC. The neoplasms were also examined for EBV DNA by the polymerase chain reaction (PCR).
Results: The mean age of patients in MC and AMC was 40.3 years (range, 27-60) and 52.0 years (range, 36-66), respectively. Positivity for *BRCA-1* was present in 68.2 % (15/22); 58.3 % (7 of 12) of MC and 80.0 % (8 of 10) of AMC. Positivity for HLA-DR was present in 59.1 % (13/22); 50.0 % (6 of 12) of MC and 70.0 % (7 of 10) of AMC. Positivity for beta-2 microglobulin was present in 72.7 % (16/22); 66.7 % (8 of 12) of MC and 80.0 % (8 of 10) of AMC. No significant differences were found in both tumor types in *BRCA-1* (p = 0.405), HLA-DR (p = 0.782) and beta-2 microglobulin (p = 0.248) immunostaining. DNA was successfully amplified using PCR, but all were negative for EBV DNA.
Conclusions: MC of the breast was similar to AMC in their expression of *BRCA-1*, HLA-DR and beta-2 microglobulin. However, these expressions were found more frequently in MC and AMC than in regular invasive duct carcinoma. MC and AMC of the breast were not an EBV-associated tumor. The relatively favorable prognosis of MC of the breast may be related to the effective tumor antigen presentation to the immune system through MHC-I (beta-2 microglobulin) and MHC-II (HLA-DR) expression.

118 Pleomorphic Apocrine Lobular Carcinoma In Situ (PALCIS): Phenotypic and Genetic Study of a Distinct Variant of Lobular Carcinoma In Situ (LCIS)

Y Chen, P Fitzgibbons, T Jacobs, G MacGrogan, H Peterse, A Vincent-Salomon, C Wa, S DeVries, E Hwang, F Waldman, S Schnitt. UCSF, San Francisco, CA; St. Jude Med Ctr, Fullerton, CA; Virginia Mason Med Ctr, Seattle, WA; Inst Bergonie, Bordeaux, France; Netherlands Cancer Inst, Amsterdam, Netherlands; Inst Curie, Paris, France; Beth Israel Deaconess Med Ctr, Boston, MA.
Background: The widespread use of screening mammography has resulted in the detection of an increasing number of breast carcinomas in situ (CIS) in which the histologic distinction between LCIS and ductal carcinoma in situ (DCIS) is problematic. We report the clinical, mammographic, pathologic, immunophenotypic, and genetic characteristics of one such lesion that we have termed PALCIS, a distinct subtype of LCIS with apocrine features that may readily be mistaken for DCIS.
Design: Ten PALCIS cases were identified during a review of problematic CIS lesions. Their clinical presentation and pathologic features were reviewed. Biomarker expression was examined using immunostaining for E-cadherin (E-cad), GCDFP-15, ER, PR, HER2, CK5/6, and Ki67. We also performed array-based comparative genomic hybridization (aCGH) using microdissected material from 2 cases.
Results: All patients were female (median age 58 yrs, range 40-86 yrs), and presented with mammographic microcalcifications. Histologically, all lesions showed distension of involved spaces by large, primarily dyshesive cells with abundant eosinophilic cytoplasm. Nuclei showed moderate to marked pleomorphism. Intracytoplasmic vacuoles were frequently present and in some instances were large enough to produce signet ring cells. All lesions showed necrosis (comedo in 9 and punctate in 1) and calcifications. All 10 cases were E-cad negative and GCDFP-15 positive, 5 (50%) were ER+, 4 of 9 (45%) PR+, 4 (40%) HER2+, 7 (70%) CK5/6+, and 7 of 9 (78%) had a Ki67 index >10%. The two cases analyzed by aCGH both exhibited loss of 16q and gain of 1q.
Conclusions: PALCIS is a distinctive form of mammary CIS, seen primarily in postmenopausal women, that may be mistaken for DCIS based on its mammographic presentation and morphologic features. While lack of E-cadherin staining and aCGH results support a relationship to classic LCIS, their histologic features and often unfavorable biomarker profile are concerning and raise the possibility that these lesions may behave more like DCIS than like classic LCIS. Clinical follow-up studies will be required to define the natural history and most appropriate management of these lesions.

119 Role of CRxA-01 and Mammaglobin Expression in the Differential Diagnosis of Paget's Disease of the Nipple Versus Extra Mammary Paget's Disease

V Chhiber, B Xu, M Retter, S Bardova, KL Rock, A Khan. UMass Memorial Medical Center, Worcester, MA; Corixa Corp., Seatle, WA.
Background: Paget's disease (PD) of the skin is a form of intraepidermal adenocarcinoma, which in the case of breast is associated with underlying ductal or lobular carcinoma. Extra mammary Paget's disease (EMPD) has similar morphology to mammary Paget's disease but a different histogenesis. In our previous study we showed that when used as a panel CrxA-01, which was identified by cDNA library subtraction, in combination with the well-characterized marker mammaglobin, at least one of these two markers is expressed in 90% breast cancer (BC). The aim of our present study was to evaluate the expression of these two markers in PD of the skin and see if they can assist in differentiating mammary PD from its extra mammary counterpart.
Design: We performed a retrospective analyses of PD in which formalin fixed paraffin embedded tissue was immunostained with antibodies to CrxA-01 and mammaglobin. In addition CK7, CK20 and CEA antibodies were also used to confirm the diagnosis. A total of 32 cases of mammary PD and 19 cases of EMPD (12 vulvar, 5 perianal, and 2 scrotal) were studied. All but 6 patients were females. In the 6 male patients 4 had perianal 2 scrotal lesions
Results: All 51 cases of PD both mammary and extramammary showed positive staining with CK7. All but 2 cases were CEA positive and only 8 cases showed positive CK 20 immunostaining confirming the morphologic diagnosis. In mammary PD CrxA-01 was positive in 22/32 (69%) and mammaglobin in 17/32 (53%); when used as a panel at least one of the 2 markers was positive in 26/32 (81%) cases; the positive predictive value when both mammaglobin and CrxA-01 are positive was 86% (12/14 cases). In the EMPD CrxA-01 was positive only in 5/19 (26%) and mammaglobin in 6/19 (32%); the negative predictive value when both mammaglobin and CrxA-01 arenegative was 69% (11/16 cases).
Conclusions: An immunohistochemical panel using antibodies against CrxA-01 and mammaglobin is helpful in differentiating mammary PD from extra mammary paget's disease.

120 Estrogen Receptor beta Expression in Myoepithelial and Stromal Cells in Benign and Malignant Breast Tissues

YJ Choi. Yale School of Medicine, New Haven, CT.
Background: Estrogen receptor(ER)-beta is expressed in many different human adult tissues at the mRNA and protein level. In breast tissues, ER-beta has been studied mostly in breast cancer and also rarely in benign breast lesions. The results are not consistent and are in fact contradictory. This may be due to the variability of detection techniques, the number and types of cells expressing the receptor, and its interpretation. In this study, we conducted ER-beta expression in benign and malignant breast tissues to delineate the types of cells expressing the receptor.
Design: FFPES of 100 cases of different types of breast cancer and 50 cases of benign and atypical proliferative lesions were subjected for ER-beta expression by the immunohistochemistry after antigen retrieval, by using antibodies to ER-beta (1:40 BioGenex, San Ramon, CA), ER-alpha(DAKO, CA) and p63(DAKO, CA) with automated DAKO immunostainer.The ER-beta expression was analyzed at each cellular components in different lesions, and compared with ER-alpha and p63 expession.
Results: While ER-ahpha was expressd only in epithelial cells, ER-beta in the nuclei of epithelial and myoepithelial (ME) cells, and in stromal cells and also infiltrating lymphocytes in both benign and malignant breast tissues. ER-beta expression in benign prolfierating ducts exhibited distinct double layered nuclear staining pattern due to ER-beta in the inner luminal glandular cell and the outer basal ME cell layers. ER beta expressed basal ME cells corresponded to the p63 stained ME cells.The distribution of ME cell is independent of duct size, length and architecture. ME cell layers were focally disrupted or attenuated in atypical proliferative lesions and carcinoma in situ, and disappeared in infliltrating carcinoma. ER-alpha and ER-beta receptors were co-expressed in both benign and malignant epithelial cells in 55 % of the cases. When co-expressed, ER-beta expression appears to be predominant
Conclusions: ER-beta was expressed not only in glandular epithelial but also in ME and stromal cells in both benign and malignant breast tissues.The presence of ME and stromal cells in different types of breast lesions and use of different techniques targeting at mRNA or protein may account for the inconsistency of ER-beta expression from other studies. As ME cells are known to play importance in cell-stromal interaction and the breast microenvironment, the potential significance of the loss of ME cells with disease progression require further study to understand the role and regulation of ER-beta in mammary carcinogenesis.

121 GATA-3 Expression Is a Predictor of Hormone Response in Breast Cancer Patients

V Ciocca, JP Palazzo, L Rose, C Daskalakis, P Parikh, R Weigel. Thomas Jefferson University, Philadelphia, PA.

Background: The expression of estrogen receptor (ER) is one of the main guiding principles to treat patients with breast cancer. However, up to one third of patients with ER positive tumors do not respond to hormonal therapy. GATA-3 is a transcription factor associated with ERα expression that plays a role in hormonal response. p53 is a tumor suppressor oncogene and its expression is associated with high-grade tumors and chemoresistance in breast cancer patients. We investigated by immunohistochemistry the expression of GATA-3 in ER positive and p53 positive and negative tumors to elucidate its role in breast cancer response to hormonal therapy.
Design: Included in the study were invasive breast carcinomas that were fixed in formalin and then paraffin embedded. 14 ER positive patients unresponsive to hormonal therapy and an age-matched control group of ER positive patients who exhibited hormone response. 5 p53 positive and 5 p53 negative cases were also studied. ER and GATA-3 were considered positive when 20% or more of the cells showed nuclear staining and p53 when more than 50% were positive.
Results: All cancers in the age-matched control group stained positively for GATA-3 expression, whereas, only 8 out of 14 (57%) tumors in the hormone unresponsive group stained positively for GATA-3. This difference was statistically significant (p=0.031) with a low odds ratio (0.122). Loss of GATA-3 expression was associated with increased risk of hormone unresponsiveness. All tumors that expressed p53 were negative for GATA-3 except for one case. p53/ER positive tumors were also negative for GATA-3. The majority of GATA-3 negative tumors were of high histologic and nuclear grades.
Conclusions: GATA-3 expression can help improve prediction of hormone responsiveness in breast cancer patients. The association between low GATA-3 expression and hormonal therapy resistance supports the role of this transcription factor as an important mediator of ERα function in breast cancer resistance. In addition, absence of GATA-3 expression may contribute to the resistance of p53 positive tumors to chemotherapy independently of ER status.

122 The Quantitative Significance of Lobular Neoplasia and Atypical Ductal Hyperplasia in Breast Needle-Core Biopsy

BZ Clark, Y Lin, DJ Dabbs. University of Pittsburgh, Pittsburgh, PA.

Background: Atypical ductal hyperplasia detected on breast needle-core biopsy is currently treated with excisional biopsy, as approximately 25% of excisional biopsies will reveal the presence of malignancy. Recently, lobular neoplasia in needle-core biopsy was associated with malignancy in subsequent excisional biopsy in 14% of cases. We investigated the association between subtypes of atypical ductal hyperplasia and the number of foci of atypical ductal hyperplasia in breast needle-core biopsy compared to lobular neoplasia, and the presence of ductal carcinoma in-situ or infiltrating carcinoma in surgical excision specimens.
Design: A search of our Copath laboratory information system for the period late 1997 to early 2003 yielded 170 cases with a diagnosis of atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), or lobular carcinoma in-situ (LCIS) on core biopsy. The cases were reviewed and cases with a follow-up excisional biopsy were included in the study. ADH was further subtyped as usual type, columnar type, or columnar type with apocrine features. The number of foci of ADH was also assessed. Fisher's exact test was used to test the association between ADH subtypes, number of foci of ADH, and lobular neoplasia in needle-core biopsy and malignancy in subsequent excisional biopsy.
Results: Of 100 cases studied, 21 needle-core biopsies showed ALH or LCIS. Ductal carcinoma in-situ (DCIS) or infiltrating carcinoma was identified in subsequent excisional biopsy in six cases (28.6%). Seventy-nine cases showed ADH, and DCIS or infiltrating carcinoma was identified in 25 cases (30.86%). Two cases showed two subtypes of ADH, columnar cell type and columnar cell type with apocrine features. There was no statistically significant difference between ADH and lobular neoplasia in needle-core biopsy and the risk of DCIS or infiltrating carcinoma in subsequent excisional biopsy (p-value close to 1). There was no difference in the risk of carcinoma in excisional biopsy between subtypes of ADH (p-value = 0.96), or with two to five foci of ADH in needle-core biopsy compared to one focus (p-value = 0.42).
Conclusions: In this study, there was no statistically significant difference in the risk of upstaging to DCIS or infiltrating carcinoma in subsequent excisional biopsy specimens related to the presence of ADH and its subtypes or ALH/LCIS on core biopsies. One focus of ADH in needle-core biopsy conferred the same risk of carcinoma in subsequent excisional biopsy as two to five foci.

123 Outcome of Patients with Ductal Carcinoma In Situ (DCIS) "Treated" by Diagnostic Biopsy Alone: Results from the Nurses' Health Study

LC Collins, RM Tamimi, H Baer, JL Connolly, GA Colditz, SJ Schnitt. Beth Israel Deaconess Medical Center; Brigham and Women's Hospital; Harvard School of Public Health; Harvard Center for Cancer Prevention; Harvard Medical School, Boston, MA.

Background: Studies of patients with DCIS "treated" by diagnostic biopsy alone have been rare, but provide important opportunities to gain insights into the natural history of these lesions and their propensity to progress to invasive breast cancer.
Design: We conducted a case-control study of benign breast disease (BBD) and breast cancer risk nested within the Nurses' Health Study. Cases were women with biopsy-confirmed BBD who subsequently developed breast cancer. Controls were women with biopsy-confirmed BBD who have not developed breast cancer, and were matched to cases on year of birth and year of benign biopsy. Slides from 1877 breast biopsies originally diagnosed as benign (371 cases; 1506 controls) were reviewed. During the course of this review, we identified 13 biopsies that contained previously undetected DCIS. Since each of these women was originally given a benign diagnosis, they received no treatment beyond the diagnostic biopsy and represent the population for this study.
Results: When compared with women with non-proliferative lesions, the odds ratio (O.R.) for the development of invasive breast cancer among women with retrospectively identified DCIS who had no treatment beyond the original diagnostic breast biopsy (n=6 cases) was 13.5 (95% CI, 3.7-49.7); the O.R. for the development of any subsequent invasive or in situ breast cancer event (n=10 cases) was 20.1 (95% CI, 6.1-66.4). Retrospective histologic review of the 13 DCIS cases revealed that the nuclear grade was low in 4, intermediate in 6, and high in 3 women. None of the lesions showed comedo-type necrosis. Invasive cancer developed among women with DCIS of all nuclear grades, including 2 with low, 2 with intermediate, and 2 with high nuclear grade lesions. Of note, all 6 invasive breast cancers (100%) developed in the ipsilateral breast (mean time to invasive cancer, 9 years) and all were of ductal type.
Conclusions: These results provide further evidence that patients with DCIS who receive no treatment beyond a diagnostic biopsy are at substantially increased risk for developing ipsilateral invasive breast cancer (>10-fold), and that an increased breast cancer risk in this setting is seen in DCIS of low, intermediate and high nuclear grades.

124 Potential Role for Tissue Microarrays for the Study of Biomarker Expression in Benign Breast Disease and Normal Breast Tissue

LC Collins, MA Rubin, SJ Schnitt. Beth Israel Deaconess Medical Center; Brigham and Women's Hospital; Harvard Medical School, Boston, MA.

Background: Tissue microarrays (TMAs) are being used increasingly as a platform to screen large numbers of invasive breast cancers using such techniques as immunohistochemistry, in situ hybridization, and fluorescence in situ hybridization. Recent studies have suggested that invasive breast cancer TMAs constructed by obtaining up to four 0.6mm tissue cores from each donor tissue block affords adequate sampling of the cancers for screening a variety of biomarkers. TMAs also represent a potentially useful platform for high throughput screening of biomarkers in benign proliferative breast lesions (BPBL) and normal breast tissue. However, the areas of interest in such cases are by their nature limited in extent, and the adequacy of lesion sampling afforded by TMA technology in this setting is a matter of concern.
Design: To address this, we constructed TMAs from 49 BPBL including proliferative lesions without (n=42) and with (n=7) atypia, and from normal terminal duct lobular units (TDLUs; n=23). TMAs were constructed by obtaining four 0.6mm cores from the area of interest for each donor paraffin block and inserting them into a recipient block using a manual tissue arrayer (Beecher Instruments). A total of 288 cores from 72 cases were arrayed in this manner. Sections cut from the TMA blocks were stained with hematoxylin-and-eosin. Each TMA slide was examined to determine the number of cores/case in which the targeted lesion was represented.
Results: Overall, the targeted lesion was present in 160 of 285 evaluable TMA cores present on the slides (56%). However, at least one of the cores in the TMA contained the lesion of interest in 60 of the 72 cases (83%). The results for normal breast tissue and for BPBL are shown in the Table:

	Normal TDLUs	Benign Proliferative Breast Lesions
# of cores with lesion	66/91 (73%)	94/194 (49%)
# of cases with at least one core with lesion	23/23 (100%)	37/49 (76%)

Conclusions: We constructed TMAs of BPBL and normal breast TDLUs using the technology most commonly employed for constructing invasive breast cancer TMAs and found that the area of interest was present on at least one TMA core in 83% of the cases. Our findings indicate that the construction of TMAs by obtaining quadruplicate 0.6mm cores from the donor tissue blocks should prove to be a useful platform for the high throughput analysis of biomarker expression in BPBL and normal breast tissue.

125 Notch1 Expression in Benign and Malignant Mammary Epithelium

GM Crisi, SS Schneider. Baystate Medical Center/Tufts University School of Medicine, Springfield, MA; Baystate Medical Center/University of Massachusetts, Springfield, MA.

Background: Notch proteins are highly conserved proteins, important for cell-fate and development. Mutated forms of Notch have been associated with mouse mammary tumors, and a role for Notch in human breast cancer has been suggested. Notch may also play a role in tumor immunosurveillance. Preliminary data suggests that deregulated expression of Notch in breast cancer cell lines can dampen the activation of neighboring lymphocytes. In this study we examined Notch expression in breast cancer progression and whether it correlated with degree of peritumoral lymphocytic infiltrates.
Design: Thirty-two formalin-fixed paraffin-embedded tissue blocks from 16 patients with a recent diagnosis of breast carcinoma were selected from the Surgical Pathology archives. Immunohistochemical studied using a polyclonal antibody to Notch1 (C-20 anti-rabbit at 1:100; Santa Cruz Biotechnology, Santa Cruz, CA) were performed, and intensity of staining and homo/heterogeneity of immunoreactivity were recorded.
Results: All cases were in females, average age 57, with no laterality predilection. All cases demonstrated benign ductal epithelium, atypical ductal hyperplasia and ductal carcinoma in-situ. Five cases demonstrated infiltating carcinoma. Peritumoral lymphocytic infiltrates were present in 10 cases. Notch1 immunoreactivity showed a cytoplasmic and plasma membrane pattern. Benign epithelium expressed Notch1, varying in intensity and distribution within each case and from case to case. Notch1 was consistently expressed in atypical and malignant epithelium, irrespective of cytologic/histologic grade, with greater intensity than that of benign epithelium. One

sentinel lymph node metastasis was also Notch1 positive. There was no difference in intensity and distribution of Notch1 immunostaining between cases with and without peritumoral lymphocytic infiltrates. Peritumoral lymphocytes did not express Notch1.
Conclusions: Notch1 is constitutively expressed in benign mammary epithelium and its expression is qualitatively increased in atypical hyperplasia, ductal carcinoma in situ and infiltrating carcinoma, irrespective of tumor grade, supporting its role in human mammary epithelial cell development and tumorigenesis. There was no correlation between age of patient, tumor hormonal status, peritumoral lymphocytes and expression of Notch1. Our current studies are investigating activation of lymphocytes in areas of Notch and Notch ligand.

126 Her2/neu FISH Amplification in Negative Immunohistochemistry Scored Breast Carcinomas

SA Dayan, JF Silverman, YL Liu, CA Smith, SE Shackney. Allegheny General Hospital, Pittsburgh, PA.
Background: The status of Her2/neu as a prognostic and predictive marker in breast cancer is usually determined by immunohistochemistry (IHC) and/or FISH in most medical centers. Only 0 to 7% of IHC negative cases (0 or 1+) show gene amplification and accordingly, breast cancer patients with these scores are usually ineligible for trastuzumab(Herceptin) treatment. We performed slide based, whole cell amplification for Her2/neu by FISH, a method that minimizes signal loss due to partial sectioning in order to better determine the FISH status of negative IHC cases.
Design: Smears were prepared from 42 breast cancer specimens having a negative (0 or 1+) IHC Her2/neu Hercept score (DAKO, Capinteria, CA). FISH were done on slide based whole cells by Vysis using gene specific probes for Her2/neu/chromosome 17. Amplification was defined by more than 15% of the cells having greater gene copy number than chromosome copy number, and aneusomy (aneuploidy) was determined by centromere counts.
Results: The results are shown in the table:

FISH/IHC Correlation

IHC	No.	Diploid	Aneuploid	Amplified
Total	42	26	7	9
Score 0	26	20	2	4
Score 1	16	6	5	5

Conclusions: Although it is reported that amplification is uncommonly seen (0-7%) using conventional FISH (AJCP 121; S33, 2004), our results show that 9/42 (21%) negative IHC cases (DAKO score 0, 4/26 (15%) and score 1+, 5/16 (31%)) demonstrated FISH amplification. Therefore, approximately 1/5 of the negative IHC cases demonstrated Her2/neu FISH amplification with slide based, whole cell methods, which possibly indicates undertreatment of these patients.

127 Electronic Reporting of Breast Surgical Pathology

NM Diaz, CE Cox, V Vrcel, S Clark, J King, K Dawson. Moffitt Cancer Center and Research Institute, Tampa, FL.
Background: Consistent reporting of surgical pathology examinations has always been important and has received special attention over the last decade. Checklists were a simple, yet important, advance in facilitating standardized reporting content.
Design: Our goal was to design a breast-pathology electronic reporting system (BERS) to further enhance reporting capabilities. A key objective was to implement a menu-driven "point and click" software program for pathologists to use in preparing specialized reports, and, at the same time, to meet the informational needs of clinicians in our cancer center's breast program. A goal of the software design was synoptic reporting, which would employ uniform nomenclature and provide standard content. BERS would also serve as a database for others in the cancer center.
Results: Our design resulted in a BERS with four key menus consisting of surgical specimen, breast diagnostic, and lymph node diagnostic data and a lymph node summary. The "discrete fields" into which diagnostic entries are digitally stored may easily be "mined" by investigators, tumor registrars, and quality improvement professionals, among others. Our BERS allows pathologists to record their observations in "real-time". The elimination of transcription turnaround time and associated costs is an added benefit. The familiarity required to use the comprehensive reporting menus of BERS is rapidly acquired by surgical pathologists. The reporting formats has been accepted as a significant improvement by clinicians. Present limitations of BERS include lack of "intelligent" menu selection capabilities, i.e. required menu selections for specific selections.
Conclusions: In conclusion, we have developed and implemented a BERS that enhances our ability to communicate throughout our clinical and research community.

128 Angiosarcoma of the Breast: Histopathologic Prognostication and Immunohistologic Features

NM Diaz, CE Cox, JO Palmer. Moffitt Cancer Center and Research Institute, Tampa, FL; St. John Medical Center, Tulsa, OK.
Background: Primary angiosarcoma of the breast (AB) is a rare, but frequently lethal, disease.
Design: We analyzed the clinical features of 18 cases of AB. All tumors were graded histopathologically without knowledge of the patients' outcome. Immunohistochemical assessment of 13 cases was done.
Results: The median age at diagnosis was 39 years. All patients were women and presented with a palpable mass. Therapy was local (surgery and/or radiation therapy) in 14 cases and systemic (chemotherapy) with or without local therapy in 4 cases. Axillary metastases were not observed. 18 patients were observed a median period of 4.8 years. 1/6, 0/2 and 7/10 patients with Grade 1, 2 and 3 AB respectively, developed a recurrence (local, contralateral breast, or disseminated disease). Patients with Grade 1 or 2 AB were less likely than patients with Grade 3 AB to develop a recurrence (by log-rank test, p=0.01). Cells lining vascular-like channels expressed vimentin, CD 34 and Factor VIII-related antigen, and reacted with Ulex europaeus lectin. This layer of cells was surrounded by type IV collagen and muscle specific actin-reactive cells. In contrast, cells within solid areas expressed only vimentin uniformly and were not associated with type IV collagen. Cytokeratin expression was not detected in either vascular or solid tumor areas.
Conclusions: We conclude that histopathologic grading of AB is a useful indicator of prognosis. The characteristic immunophenotype of differentiated vascular tumor areas is not conserved in undifferentiated solid areas.

129 Does D2-40 Immunohistochemical Staining Improve Detection of Lymphovascular Space Invasion in Invasive Breast Carcinoma When Compared to Deeper H&E Levels?

KD Doeden, N Joste, T Bocklage. University of New Mexico, Albuquerque, NM.
Background: Lymphatic invasion by breast cancer cells is an important prognostic indicator in lymph node negative patients. In addition, tumor emboli in lymphatics may herald a worse prognosis, even for individuals with node positive carcinoma. Determining lymphatic space invasion is not straightforward. Mimics such as artifactural tissue spaces around tumor may be deceptive. A recently developed antibody to the M2A antigen, D2-40, is characterized as a sensitive and specific marker for lymphatic channels. Our goal was to determine if D2-40 immunohistochemical staining is more sensitive and specific than H&E levels in predicting lymph node positivity.
Design: Specimens from 45 patients were selected comprising tumors previously diagnosed as positive or negative for lymphovascular invasion. Tumors derived from both lymph node negative and lymph node positive patients. Three H&E stained levels were examined for lymphovascular invasion from one or more selected representative tumor blocks. Also, each tumor case was stained with monoclonal antibody to D2-40 (Sigma; 1:40 dilution) using a standard avidin-biotin detection system. H&E stained levels were scored as negative or positive for lymphatic invasion. D2-40 stained tumor sections were interpreted as being positive for lymphovascular invasion if they showed complete circumferential staining surrounding tumor. Each result was independently verified by three observers. Comparison of lymphovascular invasion identified by H&E stained levels and D2-40 immunohistochemistry and correlation with lymph node status were analyzed by χ^2 statistical analysis.
Results: There was a strong positive correlation between the presence of lymphatic invasion as identified on H&E stained levels and by D2-40 staining and lymph node status (p-value <0.01 for both by χ^2 test). Examination of H&E stained levels and D2-40 immunohistochemistry detected lymphovascular invasion in 15 of 17 cases (88%) and 11 of 16 cases (69%) with documented metastatic carcinoma on lymph node dissection, respectively. One case disclosed lymphovascular invasion on H&E levels and 2 cases revealed lymphatic involvement on D2-40 immunohistochemistry with no documented metastatic disease in lymph nodes.
Conclusions: Although D2-40 has been shown to be a sensitive and specific marker for lymphatic space invasion, it does not significantly increase detection of lymphovascular invasion when compared with deeper H&E levels cut from the same tumor block.

130 Prediction of Lymph Node Status Utilizing Selective Genomic Fluorescence In Situ Hybridization Markers in Invasive Breast Carcinoma

KS Doeden, T Bocklage, C Harris, L Davis, P Doherty, P Hraber, L Tang, B Hall, I Rabinowitz, T Williams, J Hozier. University of New Mexico, Albuquerque, NM; Exagen Diagnostics, Inc., Albuquerque, NM.
Background: Lymph node metastasis is considered to be the most reliable prognostic indicator in breast carcinoma. While several factors such as grade and size of tumor may predict lymph node involvement, we investigated the correlation of genomic markers with lymph node status. With the goal of developing and implementing a clinically useful prognostic test, we utilized and improved fluorescence in situ hybridization (FISH) technology on paraffin-embedded tissue. Seventeen genomic markers predictive of recurrence in breast cancer were discovered by global mining of genome-wide RNA microarray data and high resolution DNA array comparative genomic hybridization data. These markers are clustered on chromosomes 3, 8, 9, 10, 16, 17, 19, and 20. The copy numbers of these genes were correlated with lymph node status in a group initially consisting of approximately 200 patients with invasive breast carcinoma.
Design: Breast cancer cases were selected from patients receiving care at the University of New Mexico that had at least a four-year follow up period. The cases selected included those patients with good and poor outcome and with tumors stages I through III. Tissue sections containing invasive tumor from formalin-fixed paraffin-embedded specimens were hybridized with fluorescent probes then scanned and analyzed. Probes were derived from a 32K BAC clone set that spans the entire human genome. Gene copy numbers for the 17 markers were determined by dividing the observed amplification signals by the calculated nuclear equivalents. Marker patterns were identified in the entire population and in subsets stratified by lymph node status and outcome.
Results: Of 180 patient samples analyzed, 94 had node negative disease and 86 had node positive disease. An initial analysis showed strong association between copy numbers of 11 out of 17 probes and lymph node status (p=0.00055 to p=0.049, Wilcoxon test).
Conclusions: FISH analysis of 180 cases showed strong correlation between gene copy numbers of 11 probes with lymph node status. The use of selective genomic markers on breast biopsies containing invasive carcinoma is strongly predictive of the presence of lymph node metastasis.

131 Papillary Lesions of Breast: Value of CK5/6 in Defining Epithelial Proliferations

K Donev, U Raju. Henry Ford Hospital, Detroit, MI.

Background: Papillary lesions of breast can be broadly grouped into papillomas with a biphasic growth of epithelial and myoepithelial (ME) cells and papillary carcinomas, usually devoid of ME cells. Ductal neoplasia [Ductal carcinoma in situ (DCIS) and atypical ductal hyperplasia (ADH)] involving papilloma may be difficult to distinguish from usual hyperplasia, a frequent finding in papillomas. While muscle markers only define ME cells, high molecular weight cytokeratin (HMWCK) is expressed in ME cells as well as usual hyperplasia. As HMWCK is usually negative in ductal neoplasia it has potential value in classifying epithelial proliferations in papillary lesions. We evaluated expression of CK5/6, a HMWCK, in epithelial proliferations of papillary lesions and its value as a diagnostic aid.

Design: Of 49 cases studied 20 were papillomas without atypia (10 of these had usual hyperplasia), and 12 cases were papillomas with ADH/DCIS. 17 cases were papillary carcinomas, including 6 solid papillary carcinomas. Epithelial proliferations were studied by ABC immunoperoxidase method using CK5/6 antibody. Immunostain for calponin (a muscle marker) was also performed to distinguish CK5/6 positive epithelial cells from ME cells.

Results: All 20 papillomas without atypia (100%) demonstrated prominent ME cells with both stains. Usual hyperplasia in all 10 cases was strongly CK5/6 positive. In papillomas with atypia, the areas of ADH/DCIS were CK5/6 negative in all cases (100%), while ME cells were positive. The neoplastic epithelium in all 11 cases (100%) of intraductal papillary carcinoma was negative for CK5/6 and no ME cells were identified with CK5/6 and calponin. Epithelial proliferation in all 6 cases (100%) of solid papillary carcinoma was negative for CK5/6. In 3 of these cases (50%) few ME cells were present around fibrovascular cores and had some CK5/6 positive benign cells in a pagetoid pattern and/or as scattered individual cells.

Conclusions: 1. HMW cytokeratin (CK5/6) is useful in defining epithelial proliferations in papillomas and papillary carcinomas. 2. In papillomas, usual hyperplasia is strongly CK5/6 positive, while ductal neoplasia (ADH/DCIS) is negative. 3. Papillary carcinomas are CK5/6 negative due to negative epithelial staining and absence of ME cells. 4. Solid papillary carcinomas appear to be distinct papillary lesions that may have some ME and residual benign epithelial cells.

132 Segregation of Mammographically Detected Calcifications in Stereotaxic Core Biopsies for Targeted Histologic Evaluation: Is It Necessary?

S Easley, FW Abdul-Karim, N Klein, N Wang. Case Western Reserve University, Cleveland, OH.

Background: Stereotaxic needle core biopsy (NCB) is increasingly being used for the evaluation of mammographically detected calcifications. Radiography of NCB specimens is essential to confirm the presence of calcifications within the biopsy material. To aid and direct the pathologist in diagnosis, it has been recommended that NCBs be separated into those with and those without radiographic calcifications. However, the utility of this separation has not been established.

Design: We reviewed 80 consecutive 11 g stereotaxic NCB procedures performed for mammographic calcifications. The NCBs were separated by the radiologist into those with and those without radiographic calcifications ("calcs" and "no calcs"), and embedded separately. Three H&E levels were made from each block. Histologic sections were evaluated for the presence of calcifications and pathologic diagnosis. Also noted was whether deeper sections were obtained and the impact of these deeper sections on final diagnosis.

Results: Calcifications were histologically identified in 78 of the 80 cases (98%). Calcifications were present in both the "calcs" and "no calcs" specimens in 44 cases (55%), while calcifications were present only in the "calcs" specimens in 34 (43%). The same diagnosis was rendered in the "calcs" and "no calcs" specimens in 60 cases (75%): benign/fibrocystic 32, fibroadenoma 4, papilloma 2, atypical ductal hyperplasia 6, in-situ carcinoma 13, invasive carcinoma 3. In 19 cases (24%), a pathologic diagnosis was rendered in the "calcs" specimen only (fibroadenoma 13, atypical ductal hyperplasia 4, in-situ carcinoma 2). In one case, both the "calcs" and "no calcs" specimens contained in-situ carcinoma, but invasive carcinoma was seen only in the "no calcs" specimen. Deeper sections were obtained in four cases for the purpose of finding calcifications; no change in diagnosis was made on the basis of these deeper sections, even in the three cases where deeper sections yielded calcifications.

Conclusions: The separation of stereotaxic NCBs into those with and those without radiographic calcifications does not reliably direct the pathologist to the lesion of interest, since in the majority of cases calcifications and the diagnostic lesion are found in both specimens. In rare cases, the most clinically significant lesion may be present in the "no calcs" specimen, and focusing on the "calcs" specimen may be misleading. Equal attention should be given to all cores in the setting of stereotaxic NCBs performed for mammographic calcifications

133 Eleven-Gauge Stereotactic Mammotome Needle Core Biopsy: A Novel Approach to Management of Radial Scars

M Edelweiss, V Selinko, WT Yang, E Resetkova. UT M. D. Anderson Cancer Center, Houston, TX.

Background: Radial scars (RS) are benign lesions of the breast that may mimic carcinoma on mammography. If suspected on imaging, these lesions are often managed by surgical excision because of known association with malignancy. Recently it has been advocated that extensive sampling by 14-gauge needle core biopsy could be an alternative to the surgical treatment in selected cases (Cancer 2003;97:345-51). Others suggest that this approach carries a small, but significant error rate, leading to underestimation of malignancy. Our goal was to test if larger sampling achieved by 11-gauge stereotactic needle core biopsy (11-SNCB) may have an increased sensitivity in detecting RS-associated pathology.

Design: To evaluate this hypothesis, 29 mammographically detected radial scars sampled by 11-SNCB were retrospectively examined. The histological findings of needle core biopsies and subsequent surgical excisions, if performed, were reviewed and correlated with radiological findings. Ten patients had undergone surgical excision - (S) group, and 19 patients had mammographic surveillance only - (M) group.

Results: All 29 SNCB demonstrated histological features typical of radial scar. The average number of cores was 12.1± 3.6 and 12.4± 4.3 in (S) and (M) groups, respectively. There was no significant difference between groups in terms of size of mammographic abnormality 1.5± 0.9 cm (S) versus 1.1± 0.8 cm (M). However, the presence of architectural distortion was a major radiological finding in the (S) group (70%), while the major finding in the (M) group was suspicious microcalcifications (84%). RS-associated atypia was initially seen in three of 29 cases (10%) on SNCB, including lobular neoplasia (LN), atypical ductal hyperplasia (ADH) and atypical apocrine hyperplasia (AAH). These 3 patients underwent surgical excision with no histologic upgrade seen. In 2 cases that were initially diagnosed as proliferative fibrocystic changes within RS, ALH and AAH was present on excision. No other significant pathology was observed at the periphery of surgically excised RS. Mammographic follow-up in the (M) group showed no significant findings.

Conclusions: Our study supports that 11-SNCB may have a better predictive value in identification of RS-related pathology. Extensive sampling by 11-SNCB together with careful pathologic and radiologic correlation and close clinical follow-up could potentially avoid unnecessary surgery in selected RS cases.

134 Postive Predictive Value of Diagnosis of Atypia in Breast Core Needle Biopsies

C Ersahin, S Gabram, K Yao, P Rajan. Loyola University, Maywood, IL.

Background: The diagnosis of atypical ductal hyperplasia (ADH) comprised of 1-9% breast core biopsies in the published literature. The incidence of carcinoma in the subsequent excision biopsies varied from 3.7-22% in different institutions. The aim of this study is to evaluate the reproducibility and positive predictive value of diagnosis of ADH in terms of detecting biologically significant lesions on excision biopsies.

Design: All the breast core biopsies with a diagnosis of ADH were identified during the period of 68 months from January 1999 till August 2004. All cases were reviewed by two investigators. Both quantitative and qualitative criteria were applied to distinguish low-grade ductal carcinoma in situ from ADH. In selected cases stains for Keratin (CK5/6) and mucin (mucicarmine or diastase-PAS) were performed to further characterize the lesions. Follow-up information was obtained by reviewing subsequent exicisional biopsy slides and from radiology reports.

Results: Age of the patients ranged from 32 to 95 years (mean age of 59.3 years). Of the 852 core biopsies, a diagnosis of ADH was rendered in 49 cases (5.7%). Excision biopsy diagnosis was available for 30 patients. Histological examination of excision biopsies revealed carcinoma (ductal carcinoma in situ and invasive ductal carcinoma) in 7 (23.4%), lobular carcinoma in situ in 1 (3.3%), benign phyllodes tumor in 1 (3.3%), atypical ductal hyperplasia in 9 (30%) and benign histology in 12 (40%) cases. Follow-up information by radiological surveillance was available for 11 patients who had not undergone excision biopsy. To date none of the 11 patients had significant findings on radiological observation. Eight patients were lost to follow-up.

Conclusions: The diagnosis of ADH comprised 5.7% (49/852) of core biopsies reported from our institution. Carcinoma was detected in 23.4% of core biopsies with a diagnosis of ADH. Positive predictive value of diagnosis of atypia on core biopsies is 60%. This study further emphasizes the need for surgical excision biopsy for patients with a core biopsy diagnosis of ADH.

135 Differences in the Rate of Her-2/neu Gene Amplification in Cases Reflexed from Immunohistochemistry Performed by Manual Review Versus Image Analysis

FJ Espinoza, AJ Kystoobayeva, KJ Bloom. ChromaVision Medical Systems, Irvine, CA.

Background: Her-2/neu is a receptor tyrosine kinase protein that can be quantitatively assessed by immunohistochemistry (IHC). This assessment can be performed through direct visualization of an immunostained slide by a pathologist or with the aid of image analysis software. Most pathologists routinely reflex borderline IHC results for FISH testing to assess the amplification status of the HER-2 gene. This study looked at the differences in the amplification rate of cases reflexed from manual review and image analysis.

Design: We reviewed 1299 consecutive cases received by the molecular laboratory at US Labs for HER-2 FISH testing following a previously reported HER-2 immunostain. All immunostains were performed at US Labs utilizing the HercepTest kit (DakoCytomation, Carpinteria, CA) in accordance with the manufacturer's instructions. Slides with either scored manually using the FDA approved scoring system or with the aid of ACIS, (ChromaVision, San Juan Capistrano, CA). For cases scored on ACIS at US Labs, at least six different, well preserved, randomly selected fields were analyzed. For cases scored by a submitting pathologist, IHC slides were prepared and scanned at US Labs and then burned onto a DVD which was returned to the pathologist along with the stained slides. FISH testing was performed with the PathVysion kit (Vysis, Downer's Grove, IL). Twenty cells were analyzed and signals were counted in accordance with the manufacturer's instructions. A ratio of HER-2 signals to CEP-17 signals greater than or equal to 2 was considered amplified.

Results: 496 cases (38%) were scored manually at US Labs and 20% showed HER-2 gene amplification on reflex testing. 463 cases (35.6%) were reviewed at US Labs using the ACIS and 27.4% showed HER-2 gene amplification on reflex testing. 340 cases (26.1%) were scored by the submitting pathologist using the ACIS and 24.7% showed HER-2 gene amplification on reflex testing.

Conclusions: The amplification rate detected by reflex fish testing differs depending on the screening method. Use of image analysis appears to improve the detection rate of amplified tumors, most likely by eliminating non-amplified borderline cases. Even when image analysis is used however, there is still a small difference in the detection rate of gene amplification when comparing cases scored at a reference laboratory using strict scoring criteria and those scored by local pathologists.

136 Tubulolobular Carcinoma: A Morphologic and Immunophenotypic Study of 11 Cases

NN Esposito, M Chivukula, DJ Dabbs. University of Pittsburgh, Pittsburgh, PA; Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: Tubulolobular carcinoma (TLC), classically described as an admixture of well-formed tubular glands and cords of uniform lobular-like cells, is commonly accepted as a subtype of invasive breast cancer. Whether TLC is ductal or lobular in origin, however, is controversial. We studied the immunohistological profile of this rare subset of breast carcinoma.

Design: Formalin-fixed, paraffin embedded tissues from 11 cases of TLC were retrieved from the archives of the Department of Pathology, Magee-Womens Hospital, UPMC. Tumor histopathologic characteristics, including the presence or absence of ductal carcinoma-in-situ (DCIS) and/or lobular carcinoma-in-situ (LCIS), nuclear grade, proportion of tubular and lobular patterns, and patient clinical characteristics were recorded. The tumors were studied with antibodies against E-cadherin (NCH-38, 1:300), estrogen receptor (ER) (1D5, 1:100), progesterone receptor (PR) (636, 1:200), and Her-2/neu (CB11, 1:100). The proportion of E-cadherin staining was recorded, and ER, PR, and Her-2/neu were scored by standard methods.

Results: Mean patient age was 61, and one patient had a history of contralateral ductal carcinoma. Mean tumor size was 1.05 cm. Two cases (18%) were multifocal. Histologic examination revealed three morphologic groups: 1) an even admixture of tubules and small lobular-like cells infiltrating in cords or single cells (3 cases or 27%), 2) a predominantly lobular configuration with focal areas of tubular formation (5 cases or 46%), and 3) a predominantly tubular pattern with focal lobular features (3 cases or 27%). Average nuclear grade was 2 in group one, 1.4 in group two, and 1.3 in group three. One of the 3 patients in the first group had lymph node metastases at the time of surgery (overall rate = 9%). 10 of the 11 cases (91%) had an in-situ component, in which DCIS alone (70%) or both DCIS and LCIS (30%) were present. E-cadherin was diffusely positive in both the lobular-like and tubular components in all cases. ER was positive in 11 cases (100%), PR in 7 cases (64%), and Her-2/neu in 1 case (9%).

Conclusions: Tubulolobular carcinoma is diffusely E-cadherin positive and thus appears to be a subtype of infiltrating ductal carcinoma. It is most often associated with DCIS and ER and PR positivity. Subclassification of TLC into one of three morphologic variants may be an important prognostic factor for long term follow-up of these patients.

137 Basaloid Carcinoma of the Breast: A Review of 7 Cases, with Delineation of a Possible Clinicopathologic Entity

G Falconieri, J Lamovec, S Pizzolitto. General Hospital S. Maria della Misericordia, Udine, Italy; Institute of Oncology, Ljubljana, Slovenia.

Background: Basaloid carcinoma of the breast (BCB) is an unusual neoplasm composed of basal-type neoplastic cells similar to those found in adenoid cystic carcinoma (ACC) although lacking distinctive features such as a cribriform pattern, a dual neoplastic population (epithelial-myoepithelial/basaloid), and stromal deposits of basement membrane-like material. We present a review of 7 cases of BCB with emphasis on clinical presentation and microscopic and immunohistochemical features.

Design: Seven cases of breast cancer showing overall/predominant basaloid morphology have been retrieved from a large interdepartmental series of 51 cases. Follow-up information has been obtained. A panel of antibodies was applied against several antigens including wide-spectrum keratins, cytokeratin 14, p63, ER/PR, and smooth muscle actin.

Results: Patients' age ranged from 47 to 75 years (median 58). Patients were treated with mastectomy or quadrant excision along with axillary dissection. The largest tumor dimension ranged from 1.3 to 5.5 cm (median 2 cm). Microscopically, they features sheets, nests, and cords of actively proliferating tumor cells with ovoid, hyperchromatic nuclei with inconspicuous nucleoli and scant cytoplasm. Transition into pleomorphic basaloid carcinoma and admixture with grade III sarcomatoid carcinoma was seen in 2 cases, respectively. Tumor cells were positive only for wide-spectrum keratins (7/7) and cytokeratin 14 (5/7). Axillary lymph node metastases were seen in 3 cases. At follow up (range: 6 to 126 months), 5 patients were alive, 1 with evidence of contralateral breast cancer. One patient died 6 months after surgery because of distant metastases, and 1 patient died of liver cirrhosis with no evidence of tumor 81 months after surgery.

Conclusions: This study indicates that BCB has phenotypical and immunohistochemical qualities enabling its distinction from ACC or conventional breast carcinoma with basaloid features. It may be possible segregate BCB as a clinicopathologic entity. Compared with ACC, BCB appears to be more aggressive and may entail a more guarded prognosis.

138 Comprehensive Sectioning of Sentinel Lymph Nodes in Breast Cancer: Is the Small Prey Worth a Big Hunt?

G Falconieri, S Pizzolitto, G Gentile. General Hospital, Udine, Italy.

Background: Sentinel lymph node (SLN) biopsy has gained acceptance as a less morbid alternative in breast cancer management. Although several methods have been devised to examine SLN specimens, the extent of examination and whether it should routinely include multilevel sectioning to detect micrometastases (MM) (<2.0 mm) is still debated.

Design: All "positive" SLN from 47 patients with breast carcinoma accessioned between Nov 1, 2002 and June 30, 2004 were reviewed. Formalin fixed lymph nodes were usually bisected along the longest axis and 2.0-mm thick slices were submitted in separate tissue cassettes. Each SLN block was step sectioned at 50-µm (first 15 levels), then 100-µm intervals (level 16 and over, until block exhaustion) with one section for hematoxylin-eosin (HE) and one for cytokeratin immunostaining (CKI) using antibody MNF-116. Sections were sequentially numbered in the order they were cut. Immunoperoxidase was ordered in dubious or negative cases. The findings at various sectioning intervals were compared.

Results: 49 SLN (64 tissue blocks) showed abnormal findings including: isolated tumor cells (ITC) (14 cases), MM<1.0 mm (13 cases), MM between 1.0. to 2.0 mm (6 cases), metastases > 2.0 mm (16 cases). At the protocol sectioning levels 424/967 HE (43.8%) and 185/582 CKI (31.8%) slides contained tumor deposits. The likelihood of a positive result was comparable if sections were performed at 100-, 150-, 200-, 250- or 500-µm intervals (table). No metastases > 2.0 mm were missed. 1 MM (1.1 mm focus) would have been missed at 250- and 500-µm levels on HE but not CKI slides. No new positive cases were identified in levels deeper than the 15th.

Section interval (µm)	HE+/HE total slides (%)	CKI+/CKI total slides (%)	Potential missed targets, # cases (HE/CKI)		
			ITC	MM, 0.2 - 1.0 mm	MM, 1.0 - 2.0 mm
100	193/476 (40.5)	129/323 (39.9)	2/2	0/0	0/0
150	148/338 (43.8)	62/200 (31)	5/5	2/0	0/0
200	108/248 (43.5)	47/149 (31.5)	3/10	3/2	0/0
250	82/199 (41.2)	34/119 (28.6)	2/9	2/2	1/0
500	36/95 (37.8)	18/55 (32.7)	5/11	4/2	1/0

Conclusions: These data indicate that multilevel sectioning of SLN does not add significant yield in the detection of macro- (>2.0 mm) or MM in the 1.0 – 2.0 mm range when compared to standard examination carried on initial levels. Our results support the view that "near total" SLN examination is labor intense, cost-ineffective, and of questionable clinical usefulness.

139 HER-2/neu Gene, Chromosome 17 Aberrations and Invasive Ductal Carcinoma: A Comparative Study between Young and Old Age Women

RR Fonseca, A Tomás, S André, J Soares. Instituto Português Oncologia Francisco Gentil CROL SA, Lisboa, Portugal.

Background: Abnormalities in the expression and/or copy number of the HER-2/neu gene are present in 20-30% of invasive ductal carcinoma and are associated to poor disease prognosis. Chromosome 17 aberrations are found with similar frequency and also associate to poor prognostic factors. Results on biopathological differences between breast cancer in young and old age groups are controversial, and comparison regarding chromosome 17 polisomy has not yet been done.

Design: We reviewed 38 cases of female invasive ductal breast cancer retrieved from our institution's files, grouped by age (19 cases ≤37 years and 19 cases ≥ 69 years) and then by tumor grade with an equal number of cases in each group (2 cases grade 1, 15 cases grade 2 and 2 cases grade 3). Clinicopathologic variables were collected from medical charts. For each formalin-fixed paraffin-embedded case immunohistochemistry and dual labelling fluorescence in situ hybridization with a direct labelled centromere probe for chromosome 17 together with a probe for the HER-2/neu locus was performed. Statistical analysis was done using Fisher's Exact and Wilcoxon-Mann Whitney test (for a significance level of 5%).

Results: All cases were classified as infiltrating ductal carcinomas NOS. In both groups, 17/19 (90%) cases were ER positive; regarding node status 11/19 (58%) patients ≤37 were node-positive compared to 12/19 (63%) in patients≥69. HER-2 amplification and chromosome 17 polisomy were seen in 4/19 (21%) of young women and in 5/19(26%) of the older women group. In 2/19(11%) of the latter group there was only chromosome 17 polisomy. Comparison between young and old women groups showed that the differences of all these parameters were not statistically significant.

Conclusions: 1) The frequency of chromosome 17 polisomy was similar in the two age groups for the same tumor grade. 2) Additionally, no differences were found within the same tumor grade group, regarding stage, ER, HER-2/neu amplification in the two age groups.

140 Expression of PHD (prolyl hydroxylase)-1, PHD-2 and PHD3 Is Upregulated in the Nucleus and Cytoplasm in Neoplastic Breast Disease and Nuclear PHD Expression Is Associated with the Estrogen Receptor in Invasive Breast Carcinomas

SB Fox, H Turley, L Campo, C Han, KC Gatter, AL Harris. University of Oxford, Oxford, Oxon, United Kingdom.

Background: Hypoxia inducible factor (HIF) plays a central role in environmental tumor responses. The HIF complex is composed of a heterodimer of HIF-1α and HIF-1β. In normoxia HIF-1α is unstable, being hydroxylated by one of three prolyl hydroxylases (PHDs). This results in recognition by the von Hippel-Lindau complex and allows degradation by the proteasome through ubiquitin E3 ligase. However, in hypoxia there is insufficient oxygen to follow this pathway resulting in a HIF-1α stablization and increased transcription.

Design: We have generated monoclonal antibodies to the PHDs and used immunohistochemistry to examine the pattern and level of expression of HIF-1α and the PHDs in whole tissue sections and tissue microarrays containing up to 284 invasive breast carcinomas.

Results: HIF-1α and all PHDs showed both cytoplasmic and nuclear expression in normal and neoplastic breast. Expression was stronger in in situ and invasive carcinomas compared with normal breast. HIF-1α, PHD1, PHD2 and PHD3 were positive in the nuclear and cytoplasmic compartment in 86% (150/174) and 82% (216/265), 60% (161/

270) and 44% (120/271), 52% (95/184) and 49% (138/284) and 54% (145/267) and 59% (155/265) respectively of invasive breast cancers. PHD1 was associated with PHD2 (p=0.01) and PHD3 (p=0.0001) but PHD2 did not correlate with PHD3 (p=0.21). Cytoplasmic HIF-1α correlated with cytoplasmic PHD2 and 3. Estrogen receptor (ER) was significantly positively associated with nuclear PHD1 (p=0.03), PHD2 (p=0.04) and PHD3 (p=0.02) whereas grade was significantly inversely correlated with nuclear PHD1 (p=0.005) and PHD2 (p=0.02). Nuclear PHD2 was also significantly negatively related to size (p=0.02). Univariate analysis showed that whereas HIF-1α expression was associated with a significantly shorter relapse-free (RF)(p=0.04) and overall (p=0.05) survival (OS), PHD2 expression was associated with a significantly longer RF (p=0.03) and borderline longer OS (p=0.08). Multivariate analysis confirmed HIF-1α as a poor prognostic factor.

Conclusions: This study shows that breast cancers show variation in expression of the PHDs resulting in specific patterns. Our findings suggest that their levels might be modulated by the ER tumour background, which together with microenvironment influences will effects the level of tumor HIF-1α.

141 High Density Array CGH Analysis of Sporadic and Radiation Associated Premenopausal Breast Carcinomas

J Geradts, R Varma, H Huang, A Pryshchepava, J Groth, N Nowak, D McQuaid, J Conroy, M Mahoney, K Moysich, K Falkner, G Varma. Roswell Park Cancer Institute, Buffalo, NY.

Background: The molecular pathogenesis of premenopausal breast cancer is poorly understood. The purpose of this study was to probe for molecular changes that may be linked to a specific etiologic agent, ionizing radiation.

Design: We probed for DNA copy number aberrations (CNAs) in breast carcinomas from premenopausal women from Western New York (WNY) and from Gomel, Belarus, an area exposed to fallout from the 1986 Chernobyl nuclear accident. Genomic DNA was isolated from 47 frozen tumor specimens and CGH was performed using arrays containing more than 3000 BAC clones (average resolution 1 Mb). Twenty samples were from WNY and 27 were from Belarus. Thirty-four samples were primary tumors and 13 were lymph node metastases, including 5 matched pairs. An unsupervised hierarchical clustering algorithm was employed.

Results: The average number of total CNAs per sample was 76 (37 gains and 39 losses) (range 35-134). We identified 154 CNAs (91 gains and 63 losses) occurring in more than 10% of the samples. The most common amplifications included gains at 17q21.33 (55%), at 8q24.21 (26%) including the myc gene, and at 17q21.1 (17%) including the HER2 gene. The most common deletions were at 4p14 (93%), at 17p13.2 (26%), and at 1p36.22 (26%). Belarussian tumors had more amplifications and fewer deletions than WNY breast cancers. The frequency of gains was also dependent on age and HER2 status. In the five paired primary tumor/nodal metastasis cases, we observed a larger number of discordant than concordant DNA changes. Primary tumors tended to develop more genetic losses, while lymph node metastases contained more copy number gains. Cluster analysis revealed two distinct groups of tumors: one comprised predominantly of Belarussian carcinomas and the other largely consisting of WNY cases. Fifty CNAs occurred significantly more commonly in one group versus the other, and these included some candidate signature amplifications in the radiation associated breast cancers.

Conclusions: Our study has revealed a large number of genetic aberrations in individual premenopausal breast cancer specimens, many of which had not been described before. Nodal metastases may develop at variable times and sometimes early in breast tumorigenesis. We identified a distinct CNA profile for carcinomas from a nuclear fallout area, suggesting a possible molecular fingerprint of radiation induced breast cancer.

142 Grading and Behavior of Predominantly Spindle Cell Metaplastic Breast Tumors

H Gobbi, JF Simpson, RA Jensen, SJ Olson, AD Borowsky, DL Page. Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; Vanderbilt University Medical Center, Nashville, TN.

Background: Spindle cell metaplastic tumors (SCMT) of the breast include a spectrum of lesions ranging from low-grade fibromatosis-like (FL) lesions to high grade fibrosarcoma (FS) phenotype. We compared the clinical behavior of these predominantly spindled cell tumors stratified by grade.

Design: A series of metaplastic tumors received in consultation was reviewed and 62 cases of dominant SCMT were selected. Clinical, pathological features and follow up information were analysed. Epithelial components were scanty in all cases and immunohistochemistry for cytokeratins was done to identify epithelial differentiation. Cases with bone, cartilage, and giant cells were not included. Spindle cells were graded according nuclear atypia, cellularity, and mitotic rate. Tumors were divided in two groups fulfilling strict criteria (Cancer 85: 2170, 1999): fibromatosis-like tumors (FL; n=30 cases) and fibrosarcomas (FS; n= 32 cases) .

Results: The clinicopathologic features are summarized in Table 1. All FL tumors showed bland nuclear atypia, low cellularity, and very rare mitoses. The FS group included tumors with low-grade (n=24) , intermediate (n=7), and high grade (n=1) fibrosarcoma phenotype. All tumors expressed cytokeratins, strongly in the more epithelioid cells. Patients treated by excisional biopsy or wide excision developed local recurrence in both groups (FL= 8; FS= 10 cases). No distant or regional metastases occurred in patients of FL group. However, patients from FS group presented metastases (distant= 6 cases; and lymph node= 2 cases), in spite of tumor grade.

Conclusions: SCMT of the breast can be separately subtyped and graded. The biological behavior is defined by the major histologic phenotype. The lowest-grade lesions with a dominant fibromatosis-like phenotype behave similarly to fibromatosis with tendency of local recurrence. The fibrosarcoma-like phenotype implies a sarcoma-like behavior, including local recurrence, direct extension, as well as distant hematogenous metastatic potential, and rare lymph node involvement.

Clinical and pathological features of metaplastic tumors

Group	Average age (range) years	Average follow up (range) months	Local recurrence (n/%)	Distant Metastases (n/%)	Average tumor size (range) cm
Fibromatosis-like (n= 30)	63.4 (40-80)	38.5 (8-72)	8 (26.7%)	0	2.9 (1.2-7.0)
Fibrosarcoma (n= 32)	67.4 (36-89)	27.8 (3-72)	10 (31.2%)	6 (18.7%)	3.1 (0.5-6.0)

n= number of cases

143 Molecular LOH Clonality Determination of Ipsilateral Breast Failure Invasive Carcinomas in Patients Treated with Breast Conserving Therapy Identifies the Subset at Increased Risk for Distant Metastases

NS Goldstein, FA Vicini, S Hunter, S Forbes, LJ Kestin. William Beaumont Hospital, Royal Oak, MI.

Background: Patients who sustain an ipsilateral breast failure (IBF) following invasive breast carcinoma treated with breast conserving therapy are at increased risk of distant metastases. The risk of distant metastases does not appear to be uniform and the underlying mechanisms are unclear. Criteria to identify the group at highest risk for DM following IBF are vague. We examined the clonality relationships of IBF carcinomas in 29 patients that included six patients who developed distant metastases (DMs) subsequent to the IBF to study these issues.

Design: 29 patients with AJCC stage 1 or 2 invasive breast carcinomas treated with breast conserving surgery and radiation therapy with an IBF and no distant metastases prior to the IBF were studied. Six patients developed DMs subsequent to the IBF. Carcinoma DNA, extracted from paraffin blocks was analyzed with 20 markers. LOH was +/- 50% allelic loss relative to the allelic ratio of normal tissue. Fractional allelic loss % (FAL) (markers with LOH/ informative markers)was computed for each carcinoma.

Results: Five of the six patients (83%) who developed DMs subsequent to the IBF had clonally related IBFs. In the subset of clonally related IBF cases, the FAL was higher in the IBF than in the corresponding initial carcinoma in 15 (79%) cases. The mean increase in IBF carcinoma FAL in cases associated with subsequent DMs was +18.5% compared to a mean increase of 7.3% in IBF carcinomas not associated with DMs ($p = 0.002$).

Conclusions: Patients with clonally related IBFs comprise the main pool in which subsequent DMs occur. Clonally related IBFs generally had higher FALs than the corresponding initial carcinoma, reflective of genetic instability and progression. Not all clonally related IBFs appeared to have the same biologic risk of distant metastases. IBFs associated with DMs had significantly larger gains in their FAL than in IBFs cases unassociated with DMs, possibly reflecting a greater degree of genomic instability. Clonally different, second carcinoma IBFs appear to function independently relative to their initial carcinoma in regards to their metastatic risk. Molecular clonality assays appear to be a reliable method of identifying the patients who may maximally benefit from systemic chemotherapy at the time of IBF.

144 Molecular Clonality Assay Determination of Ipsilateral Breast Failure Invasive Carcinomas in Patients Treated with Breast Conserving Therapy: Comparison with Clinical and Biologic Factors

NS Goldstein, FA Vicini, S Hunter, E Odish, LJ Kestin. William Beaumont Hospital, Royal Oak, MI.

Background: We established the clonality of ipsilateral breast failure (IBF) invasive carcinomas relative to the initial invasive carcinomas using a PCR-LOH molecular assay to investigate clinical and biologic issues of IBFs.

Design: DNA from initial and IBF carcinomas from 30 patients with AJCC stage 1 or 2 invasive breast carcinomas treated with breast conserving surgery and radiation therapy was extracted from paraffin blocks, analyzed with 20 markers. LOH was +/ - 50% allelic loss compared to normal tissue allelic ratios. Morphologic and clinical features were evaluated in the context of IBF clonality.

Results: 19 of the 30 IBFs (63%) were clonally related to the initial carcinoma, 10 were (33%) clonally different, second primary carcinomas, and one case had indeterminate results.

Clonally Related IBFs: The morphology of the IBF and initial carcinomas were similar in 17 (89%) cases. The IBF carcinoma was the same grade as the initial carcinoma in 11 (58%) cases, one grade higher in 7 (37%) cases, and two grades higher in one (5%) case. The excision margins around initial invasive carcinomas were positive or near-greatest amount in only 5 (26%) cases.

Clonally Different IBFs: The morphology of the IBF and initial carcinomas were similar in 8 (80%) cases. The IBF carcinoma was the same grade as the initial carcinoma in 3 (30%) cases.

Factor Comparison: The IBF clinical classification differed from the molecular clonality assay result in 13 of the 29 cases (45%). Of the 10 clonally different IBF cases, 7 were clinically classified as TR/MM failures. The mean IBF time interval in clonally related and different IBF cases was 4.3 yrs and 8.7 yrs (p=0.010). The mean IBF interval was significantly shorter in higher grade carcinomas only in clonally related IBFs ($p = 0.026$) but not among clonally different IBF cases.

Conclusions: Molecular LOH assays can accurately establish the clonality of most IBFs. Morphologic comparison and clinical classification were unreliable methods of determining clonality. Two similar appearing, closely situated carcinomas can be clonally unrelated neoplasms. Positive resection margins around the initial carcinoma was not an underlying factor in most clonally related IBFs. The mean IBF time interval was significantly shorter in clonally related IBF cases, supporting others suggestion that longer time periods are needed for new second carcinomas to develop and manifest.

145 A New Anti-ER Rabbit Monoclonal Antibody Improves Efficiency of Immunohistochemical Evaluation of ER Status in Breast Cancer

AM Gown, TS Barry, P Kandalaft, LC Goldstein, CC Tse, DO Treaba. PhenoPath Laboratories and IMPRIS, Seattle, WA.

Background: Immunohistochemistry (IHC) has become the standard for assessment of estrogen receptor (ER) status in breast cancer, since it predicts clinical outcome and respose to adjuvant endocrine therapy. False negative IHC studies may result from sampling error in small needle core biopsies, or in cases where there has been antigenic compromise. Thus it is imperative that IHC studies employ the most robust anti-ER antibodies available, e.g., those displaying maximal sensitivity and efficiency, the latter defined as the percentage of cases showing high level immunostaining. To this end, we tested the relative sensitivities and efficiencies of two anti-ER antibodies: 1D5, a mouse monoclonal antibody in wide use, and SP1, a new rabbit monoclonal anti-ER antibody.

Design: This multi-institutional, retrospective study incorporated both needle core and lumpectomy specimens, from numerous geographically diverse pathology laboratories, and included a total of 9,110 breast cancer cases immunostained with the 1D5 antibody and 2,271 nonoverlapping cases immunostained with the SP1 antibody. IHC was performed following optimized epitope retrieval, with detection using a polymer based immunoperoxidase system (Envision-plus, Dakocytomation). Scoring was performed as: Negative (< 1% positive); 1+ (1-25% positive); 2+ (25-75% positive); 3+ (> 75% positive).

Results: The overall positivity rate for 1D5 was 66.7%, compared with 66.8% for SP1. Notably, there were significant differences in distribution among 1+, 2+, and 3+ rates: 1D5 showed 1+, 2+ and 3+ positivity rates of 3.53%, 12.1%, and 33.3%, while SP1 showed 1+, 2+ and 3+ positivity rates of 5.15%, 5.94%, and 55.7%, respectively.

Conclusions: In this retrospective series of 9,110 cases analyzed with the 1D5 anti-ER monoclonal antibody and 2,271 cases analyzed with the SP1 anti-ER antibody, SP1 showed greater efficiency in identifying positive cases. Specifically, there was a significantly higher fraction of positive cases falling into the 3+ category, with a reduction in the number of 2+ cases. The small but significant increase in the number of 1+ cases may reflect the increased sensitivity of the SP1 antibody. These data suggest that the rabbit monoclonal SP1 antibody may prove more efficacious in identifying ER-positive breast cancer cases in settings of low level positivity.

146 pAKT/PI3K Overexpression May Predict Poor Survival in Patients Treated with Herceptin for 3+ HER-2/neu Positive Breast Carcinoma

R Gupta, RStJ Ricketts, S Marconi, CN Otis. Baystate Medical Center/Tufts University School of Medicine, Springfield, MA.

Background: Although Herceptin is effective in treatment for some breast carcinomas that over express HER-2/neu, there are patients with tumors that over express HER-2/neu who do not respond to Herceptin therapy. Activation of protein kinase signaling pathways at alternate sites may account for Herceptin resistance. The pAKT/PI3K (phosphoinositide 3-kinase) pathway is implicated as a major alternate pathway, which up regulates cell survival/carcinogenesis even after Herceptin inhibition of HER-2/neu activation. This study evaluated the over expression of pAKT/PI3K pathway and its down stream regulators (mTOR, pBAD) to disease free survival in breast carcinoma patients treated with Herceptin.

Design: Eight cases of HER-2/neu (3+) breast carcinoma treated with Herceptin were retrieved from the surgical pathology files of Baystate Medical Center. A tissue microarray (TMA) was created using a Beecher manual tissue arrayer. Multiple components of pAKT/PI3K pathway were evaluated using antibodies to pAKT(Ser473), mTOR, (ser2448) pBAD(ser112)(7E11) and IGF(Tyr1131)/(Tyr1146) (Cell Signaling Technology), on a DAKO automated platform.

Results: Three of the 8 patients (38%) demonstrated over expression of pAKT/PI3K. These 3 patients had a 50% lower median survival time (3 yrs.) when compared to the 5 cases without over expression of pAKT/PI3K (median survival of 6.2 yrs.). Two of the 3 patients with over expression of pAKT/PI3K had activation of mTOR and one of those had activation of IGF, mTOR, and pBAD. The 5 cases without over expression of pAKT/PI3K did not demonstrate over expression of mTOR, pBAD and IGF (except in 1 case with expression of IGF).

Conclusions: The activation of pAKT/PI3K pathway predicts a worse survival in patients with breast carcinoma over expressing HER-2/neu treated with Herceptin.

147 Correlation of Her2/Neu Testing Using Chromogenic In Situ Hybridization (CISH) with Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in Breast Cancer

WM Hanna, K Kwok. Sunnybrook & Women's College Health Sciences Centre, University of Toronto.

Background: Testing for the Her2/neu status for breast cancer has become necessary, both for prognosis and as a predictor of response to chemo, hormonal and Herceptin therapy. Currently the two main methods used are IHC and FISH. Most testing algorithms recommend first screening with IHC and then use FISH for confirmation of equivocal results. FISH is currently the gold standard since 96% of cases of over-expression of the Her2/neu oncoprotein are associated with amplification of the Her2/neu gene. However, FISH is an expensive method that is time consuming, requires specialized microscopy and does not allow differentiation of the in situ from the invasive component. CISH has recently been adapted to test for the Her2/neu gene amplification using DAB as a chromogen. This allows the use of light microscopy and the assessment of the tumor morphology. Thus, CISH can be an alternative method to assess for Her2/neu gene amplification.

Design: We first assessed the CISH methodology using 2 groups of well characterized tumors. Group 1 = 39 cases which are IHC (HercepTest) negative/FISH negative. Group 2 = 38 cases which are IHC 3+, 34 were FISH positive and 4 were FISH negative. CISH was considered positive when there were 6 or more signals for the Her2/neu gene. The results between FISH and CISH were concordant in 100% of the cases in group 1 and 97.4% in group 2. We then applied the FISH and CISH testing methodology in a subset of 107 equivocal cases.

Results: In this cohort of equivocal cases, 64 cases had a FISH score of < 2 and CISH score < 6 signals (not amplified). Thirty four cases (30%) had a FISH score of ≥ 2 and CISH signals of ≥ 6 (amplified). There were 9 discordant cases. This is a concordance rate of 91.6% (98/107).

Conclusions: This data shows the high level of concordance between FISH and CISH in assessing Her2/neu gene amplification. Thus CISH could be used as an alternative method for Her2/neu gene amplification, allowing the wider availability of this test for labs that are not equipped or trained for fluorescence analysis. However, since CISH is also an expensive test, it should not replace IHC screening but only as an alternative to FISH in equivocal cases.

148 The Significance of c-kit Expression in Phylloides Tumors

WM Hanna, B Djordjevic, HJ Kahn. Sunnybrook & Women's College Health Sciences Centre, University of Toronto, Toronto, ON, Canada.

Background: A recent study has shown focal expression of c-kit, a protooncogene that encodes a transmembrane tyrosine kinase growth factor receptor (CD117) in phylloides tumors. This receptor has been demonstrated in normal epithelium and hyperplastic lesions of the breast whereas only 10% of in situ and invasive carcinomas are positive for c-kit.

Design: The aim of this study was to assess the expression of CD117 in fibroepithelial lesions of the breast. Immunohistochemical staining was performed on 34 benign, 8 borderline and 9 malignant phylloides tumors as well as 24 fibroadenomas, some with cellular stroma.

Results: C-kit positivity was noted in the cytoplasm of the epithelial elements in all cases. However, there was no positivity for c-kit in the stroma in any of the tumors.

Conclusions: This study illustrates the maintenance of c-kit expression in the epithelial component which is benign in all of these lesions. The absence of c-kit in the stromal elements indicates that c-kit does not play a role in the pathogenesis of fibroepithelial lesions including phylloides tumors. Therefore, the newly developed tyrosine kinase inhibitors would not be valuable in the treatment of phylloides tumors.

149 Automated Quantitative Analysis (AQUA) of E-Cadherin Expression in Breast Cancer Lymph Node Metastases Is Predictive of Survival

M Harigopal, AJ Berger, HM Kluger, DL Rimm. Yale University School of Medicine, New Haven, CT.

Background: E-cadherin (E-cad) is responsible for epithelial cell-cell adhesion, and it has been extensively studied in primary breast cancer. Studies have generally shown that decreased expression is associated with poor patient outcome. It is presumed that with metastasis E-cad is either lost or down-regulated, and there is very little data on E-cad expression levels in lymph node metastases.

Design: Membrane expression of E-cad was studied in primary tumors and matched nodal metastases using AQUA, an objective method of quantitative analysis of immunofluorescent protein staining. E-cad expression was evaluated using a breast cancer tissue microarray comprising 280 primary invasive ductal carcinomas (IDC) with matched nodal metastases, with 20 year follow-up. There were 207 primary IDC and 222 nodal metastases that were suitable for analysis by AQUA.

Results: The mean staining intensity of E-cad in IDC primaries and matched lymph nodes was compared by paired t test, and was not significantly different (P= 0.8). A scattergram comparing the differences in the mean staining intensities of E-cad expression between primaries and nodal metastases revealed a group of patients with higher E-cad in the node than in the primary tumor. We divided the top 25% of E-cad nodal expressers from the remainder of the cohort. This group showed significantly improved survival by Kaplan-Meier analysis (p=0.028). E-cad expression in nodal metastasis was a significant predictor for survival by Cox univariate analysis (P= 0.007), yet E-cad expression in the primary tumors was not (P=0.13). By multivariate analysis, E-cad expression in nodal metastases retained its independent predictive value, as did tumor size, nuclear grade, nodal number, race and HER 2/neu status.

Conclusions: Strong E-cad expression in lymph node metastases was highly predictive of improved survival. This suggests that re-activation or up-regulation of adhesion molecules at metastatic sites portends less aggressive tumor behavior.

150 Axillary Lymph Node Dissection(ALND) in Patients with Positive Sentinel Lymph Node(SLN): Is It Needed?

S Hart-Gouleau, D Yee, T Tuttle, M Roychowdhury, C Lee, D Aslan, S Pambuccian, E Gulbahce. University of Minnesota, Minneapolis, MN.

Background: ALND has been the gold standard for staging which affects regional control, determines prognosis and is used to determine systemic hormonal/chemo therapy(SHCT) and axillary radiation therapy(AXRT). However, major studies have failed to show a survival advantage to ALND. Thus, the benefit of ALND in patients with positive (+)SLN has been questioned especially since SLN is the only +ALN in >50% of cases. Tangential field radiation therapy may cover level I/II ALN and be used in patients with <4 +ALN. ACOSOG Z0011, the clinical trial to address these issues, has been facing difficulty in patient accrual. The aim of our study is to determine if information gained at ALND will change decisions for SHCT and AXRT in +SLN patients.

Design: Patients who underwent SLN biopsy 6/97-6/04 were identified. Age, tumor and metastases characteristics (tumor size, type, grade, ER/PR, HER2/Neu status, lymphvascular invasion(LVI), status of SLN and ALND, size of metastasis, detection method, extracapsular extension(ECE) were reviewed. A medical oncologist was

provided with all the information and asked to decide on SHCT and AXRT based on SLN only, and then with the knowledge of ALND.
Results: 62/227(27%) of patients (av. age:53; range:30-78) had +SLN. Average SLN and +SLN was 1.9(1-5) and 1.1(1-2) respectively. 55 patients had all information available for SHCT decision. In 14/55 of the cases tumor in SLN was initially identified on IHC. ALN staging showed: N0(i+):12; N1mi:13; N1a:22; N2a:8. 1/41(2.4%) cases in which the SHCT decision was based on SLN (H&E) required a different SHCT with the knowledge of ALND. All 62 patients with +SLN were evaluated for AXRT. 8/227(3.5%) of all patients and 8/62(13%) of patients with +SLN required a change in AXRT based on ALND. However, in 5/8(63%) of cases presence of >3 +ALN could be predicted by presence of ≥3/5 morphologic predictors of non-SLN metastasis: tumor>2cm, metastasis>1cm, no.of +SLN>1, ECE, and LVI.
Conclusions: The need for ALND in patients with +SLN to make treatment decisions is questionable. Only 1/41(2.4%) of patients with +SLN and 1/227(0.4%) of all patients required a different SHCT based on ALND. While ALND may be useful to predict the need for AXRT, histological features of the +SLN and the tumor may be used to determine patients with >3 positive nodes. With this information, only 3/62(4.8%) of +SLN and 3/227(1.3%) of all cases required a change in AXRT(other than level I/II) which were comparable to the false negative rate of SLN biopsy.

151 The Expression of the Focal Adhesion Protein Paxillin in Breast Cancer Correlates with *HER2* Amplification and May Help Predict a Better Response to Chemotherapy

D Hicks, B Yoder, S Short, E Tso, T Choueiri, G Budd, J Crowe, T Morken, M Skacel, P Roche, T Grogan, S Tarr, R Tubbs. Cleveland Clinic Foundation, Cleveland, OH; Lab Vision Corp., Fremont, CA; Ventana Medical Systems, Inc., Tucson, AZ.
Background: Activation of heregulin (*HRG*) signaling has been implicated in the development of an aggressive phenotype in breast cancer. Paxillin (PAX), a cytoskeletal focal adhesion protein is transcriptionally up regulated and phosphorylated by *HRG* treatment of tumor cells in vitro. PAX expression may correlate with *HER*2 amplification in breast cancer patients as well as clinical response to Herceptin™ treatment in combination with chemotherapy. In the current study, we examined the relationship between PAX expression and pathologic features, prognostic markers, clinical outcome, and chemotherapy response in patients with invasive breast carcinoma.
Design: Tissue microarrays containing 313 well-characterized primary invasive breast carcinomas (average follow up 60 months) were assessed for PAX expression (Lab Vision) via IHC. PAX staining was compared to: ER/PR, *HER*2 status, tumor size, histologic grade, nodal status, disease free (DFS) and overall survival (OS).
Results: PAX reactivity was seen in 27.8% (87/313) of breast carcinomas and demonstrated a diffuse cytoplasmic pattern of staining. PAX expression correlated with *HER*2 gene amplification by ISH (p=0.004) and there was a trend toward shortened OS and DFS among PAX (+) patients (p=0.1). No correlation was found between PAX expression and ER/PR, grade, size, or lymph node metastases. Among the 15 HER2 (+) patients receiving chemotherapy, 6 of 6 *HER*2 (+)/PAX (-) patients recurred, while only 4 of 9 (55.5%) *HER*2 (+)/PAX (+) patients demonstrated a recurrence (p=0.01). Additionally, an improved overall survival was seen for *HER*2 (+)/PAX (+) patients (9/9) compared with HER2 (+)/PAX(-) patients (3/6, 50%)(p=0.01) after chemotherapy.
Conclusions: Investigation of the over-expression of genes that are transcriptionally up regulated by important signaling pathways in breast cancer may add to our understanding of the clinical course of disease and aid in selecting appropriate therapies. This data suggest that PAX up-regulation may be an integral part of the *HER*2 pathway in breast cancer. PAX expression may also identify a subset of *HER*2 (+) breast cancer patients more likely to respond to chemotherapy. Further study of a role for PAX expression in predicting response to cytotoxic regimens or targeted treatments is warranted.

152 Expression and Initial Characterization of the Tetraspanin Superfamily Member NET6, a New Candidate Tumor Suppressor Gene, in Breast Cancer Cells In Vitro and In Vivo

H Huang, J Groth, D Fleming, K Sossey-Alaoui, R Sawhney, L Hawthorn, J Geradts. Roswell Park Cancer Institute, Buffalo, NY.
Background: NET6 is a new and uncharacterized member of the tetraspanin family of cell surface (glyco)proteins. Other members of this family such as KAI1/CD82 and CD9 have known tumor or metastasis suppressor function. In previous gene expression profiling experiments, we identified NET6 as one of about 300 genes that were differentially expressed in HER2-positive versus HER2-negative breast cancer cells.
Design: Real time quantitative RT-PCR was used to detect NET6 mRNA expression in 9 breast cancer cell lines and in 34 frozen breast carcinomas. Western blotting and immunohistochemistry (IHC) were used to detect NET6 protein level, using a custom made rabbit polyclonal antibody. DNA transfection was used for stable NET6 overexpression in MDA-MB-231 cells. Changes in morphology, growth rate and cell migration were examined.
Results: By quantitative PCR (expression relative to GAPDH), NET6 levels were significantly higher in the HER2-positive breast cancer cell lines (403 vs. 71, p=0.033). Moreover, HER2-positive breast carcinomas also showed elevated NET6 expression (793 vs. 438, p=0.049). A larger difference in NET6 levels was observed between ER-positive and -negative tumors (965 vs. 215, p<0.001). The differential expression of NET6 in HER2-positive versus -negative carcinomas was more pronounced in the ER-negative group (p=0.004) and failed to reach significance in the ER-positive group (p=0.31). NET6 expression was inversely correlated with tumor grade. The differential expression of NET6 at the protein level was confirmed by Western blotting and IHC. Stable transfection of NET6 into low expressing MDA-MB-231 cells induced changes in morphology, reduced motility and significantly decreased the growth rate.
Conclusions: This is the first study implicating the new tetraspanin NET6 in human mammary neoplasia. We confirmed its elevated expression in HER2-positive breast cancer cells, both in vitro and in vivo. However, ER-negative and high grade tumors had markedly decreased NET6 levels. Moreover, transfection of NET6 into MDA-MB-231 cells had a significant inhibitory effect on proliferation and cell migration. These findings suggest that NET6 may be a novel candidate breast cancer suppressor gene.

153 Morphologic Features of Ipsilateral Recurrent Breast Cancers in Patients Treated with Breast Conserving Therapy (BCT)

L Huo, Y Wu, F Meric, AA Sahin. M.D. Anderson Cancer Center, Houston, TX.
Background: For many invasive breast cancers, BCT consisting of segmental resection followed by radiation therapy has replaced modified radical mastectomy. However, a considerable number of these patients develop ipsilateral recurrence. In this study, we reviewed morphologic features of post-BCT recurrent tumors.
Design: Thirty-six cases with available slides for review were identified for this study. All patients had invasive breast cancer treated by BCT at M. D. Anderson between 1981 and 1996, and subsequently had ipsilateral recurrence. The interval between the primary and the recurrent tumors ranged from 1.1 to 16.7 years, with a mean interval of 4.7 years. Morphologic features including histological type and grade, tumor necrosis, lymphovascular invasion, and associated DCIS were evaluated.
Results: The morphologic features of the 36 recurrent tumors are summarized in Table 1. In addition, 17 cases had slides of the primary tumors available for comparison. Twelve of the 17 cases showed identical/similar morphologic features between the primary and recurrence, including 11 invasive ductal ca. and 1 mixed ductal and lobular ca. Of these 12 cases, 6 were associated with DCIS. The 5 cases showing different morphology included 1 being ductal in the primary and mixed ductal and lobular in the recurrence; 1 mixed ductal and lobular in the primary and ductal in the recurrence; 1 predominantly glandular in the primary and solid in the recurrence; 2 with lower nuclear grade in the recurrence than in the primary.
Conclusions: The recurrent tumors studied here demonstrate heterogeneous morphologic features. Five of 17 cases show a different morphology between primary and recurrent tumors, raising the possibility of these being second independent tumors. This is also reflected by the finding that 75% of the recurrent tumors have associated DCIS, including 50% of those that show identical/similar morphology to their primary tumors. Thus, the genetic background may play a key role in these recurrent tumors. Further studies by molecular approaches may help elucidate the mechanisms of post-BCT recurrence.
Table 1. Morphologic features of recurrent tumors.

Type	Number	Tumor necrosis	Lym-vas. invasion	Tumor grade 1 / 2 / 3	Assoc. DCIS	DCIS necrosis	DCIS grade 1 / 2 / 3
IDC	30	4	4	2 / 14 / 14	22	15	0 / 13 / 9
MIXED	5	0	1	0 / 5 / 0	4	3	1 / 2 / 1
ILC	1	1	0	0 / 1 / 0	1	0	1 / 0 / 0
TOTAL	36	5	5	2 / 20 / 14	27	18	2/ 15/ 10

154 Absence of Heterotopic Non-Neoplastic Epithelial Tissue in 3904 Axillary Lymph Nodes Studied by Serial Sectioning

S Iken, M Schmidt, SC Schaefer, CJ Kirkpatrick, HA Lehr. University of Mainz, Mainz, Germany.
Background: The sentinel node approach to axillary dissection is increasingly being integrated into routine surgery of breast cancer. However, even with meticulous work-up of sentinel nodes during frozen section diagnosis, limitations of serial sectioning and section quality result in occasional cases where nodal metastases are missed during frozen section and only later identified during work-up of paraffin-embedded sections. Hence, some groups/companies are promoting the use of rapid PCR using cytokeratin primers for the detection of metastases in sentinel lymph nodes. Yet, non-neoplastic heterologous breast tissue may occasionally be present within axillary lymph nodes, and these epithelial cells will be amplified and the signal interpreted as metastatic tumor cells. The consuming nature of PRC technology precludes verification of this diagnosis by subsequent histology and these patients will be subjected to unnecessary axillary dissection and to aggressive adjuvant treatment. While the presence of non-neoplastic breats tissue has been well recognized in many case reports, not a single study has ever tried to assess the frequency of such heterologous implants as a basis of adequate patient counseling.
Design: During the autopsy of 34 female and 46 male unselected patients, bilateral axillae were dissected under supervision of a surgical gynaecologist (M. Schmidt). A mean of 25±11 lymph nodes per axilla were dissected in levels 1 (16±7), 2 (7 ±5) and 3 (2±2). In total, 3904 lymph nodes were serially sectioned at 100 µm increments and 4µm sections stained with H&E according to standard protocols. In about one third of cases (n=1102 lymph nodes), adjacent sections of all H&E sections were also stained with pan-cytokeratin antibodies by IHC.
Results: In not a single one of 3904 lymph nodes could we identify non-neoplastic heterologous epithelial tissue, neither by H&E stains, nor by IHC using pan-cytokeratin antibodies.
Conclusions: Even though this study is characterized by a striking disequilibrium between the enormous experimental effort and the conciseness of the obtained data, we believe that the documentation of absence of non-neoplastic breast tissue in almost 4000 meticulously analysed axillary lymph nodes should provide a sound basis for the estimation of the risk of false positive PRC results in sentinel node work-up and should hence be invaluable for the counseling of patients undergoing surgery for breast cancer.

155 Loss of Heterozygosity Patterns in Flat Atypical Lesions of the Breast

KV Inamdar, M Roemer, CA Abbud, I Newsham, UB Raju. Henry Ford Hospital, Detroit, MI.

Background: Flat atypia/clinging carcinoma, originally described by Azzopardi, consists of dilated ducts lined by stratified epithelial cells showing columnar or rounded cell change with cytologic atypia. It often coexists with the more conventional forms of atypical ductal hyperplasia (ADH) or ductal carcinoma in situ (DCIS) and tubular carcinoma. There is controversy regarding the benign versus malignant nature of such lesions and their management. As the morphologic criteria continue to be elusive, molecular genetic analysis may provide some insight into their biological continuum with ductal neoplasia. We intend to characterize these lesions and examine whether the genetic events underlying them are similar to the conventional forms of ADH and DCIS and thus provide evidence of their pre-invasive nature at the molecular level.

Design: Fifteen cases including 12 pure flat atypical lesions (PFL) and one case each of tubular carcinoma (TC), ADH and DCIS were examined. Paraffin sections (10 um) were treated with xylene and ethanol and luminal epithelial cells isolated with the PixCell IIe Laser Microdissecter. DNA was extracted from normal and tumor cells and subjected to PCR with seven polymorphic markers on three chromosomal regions (Ch) which show significant loss of heterozygosity (LOH) in breast cancer including 3p25-24, 3pter-25, 9p23-22, 9p21 and 11p15.5. PCR products were analyzed on the CEQ 8000 DNA Fragment Analyzer. A greater than 30% loss in intensity as measured by the area under the curve of the allelic peak of one allele was considered to represent LOH.

Results: Eleven out of 15 cases (73%) showed LOH in at least 1 marker on Ch 3, 6 cases (40%) on Ch 9 and 4 cases (27%) on Ch 11. LOH was most frequent at the locus 3p25 (67% cases) followed by 11p15.5 (40%) and 9p21 (53%). For PFL, 9 out of 12 (78%) cases showed LOH at the locus 3p25, 7 cases (60%) at 9p21 and 6 cases (50%) at 11p15.5. Overall, PFL cases showed LOH in at least 1 marker on Ch 3 in 64% of cases, on Ch 9 in 38% of cases and on Ch11 in 22% of cases. Combined frequency of LOH for PFL in at least 1 marker was 44%. TC showed LOH at the same locus (3p25) as in PFL. DCIS case showed LOH at 9p21 locus also seen in 60% of PFL.

Conclusions: Flat atypical lesions are frequently associated with LOH at loci that harbor putative tumor suppressor genes. These alterations are similar to those found in the more conventional types of DCIS and tubular carcinoma. The data supports neoplastic continuum of these lesions with ductal neoplasia.

156 Intraductal Papillomas without Atypia on Breast Core Needle Biopsy (CNB): Surgical Excision Is Advisable

TW Jacobs, DG Guinee, J Holden, B Hashimoto, D Wechter. Virginia Mason Med Ctr, Seattle, WA; Univ. of Utah, Salt Lake City, UT.

Background: The management of patients with a diagnosis of intraductal papilloma without atypia (PapWA) on breast CNB is controversial. Of concern is whether the features observed on CNB are representative of the most worrisome area of the targeted lesion, since carcinoma or atypical hyperplasia may only focally involve an otherwise benign papilloma. Some recent reports have suggested that surgery for PapWA on CNB is unnecessary, however all have had few patients with excision follow-up. A larger series of PapWA on CNB would be more sensitive to determine prevalence of carcinoma or atypia at excision and may identify features predictive of surgical outcome.

Design: We therefore analyzed the histologic features of 38 consecutive cases of PapWA on CNB, all with excision follow-up. At least 2 pathologists reviewed all slides and independent agreement was required regarding the absence of atypia on CNB. CNB were immunostained for CK5/6, p63 and the proliferation marker topoisomerase 2α (Topo). CNB histology, immunostains, imaging and excision histology were reviewed in a blinded fashion and the results were then correlated.

Results: On excision, 3 cases had ductal carcinoma in situ (DCIS), 3 had atypical ductal hyperplasia (ADH) and 3 had lobular neoplasia (ALH/LCIS). DCIS was within and adjacent to the excised papilloma in 2 cases and close in 1 case. ADH was adjacent to the papilloma in 2 of 3 cases. The percentage of CNB involved by PapWA was significantly lower for cases with ADH or DCIS at excision (Med 20%, range 10-80%) vs those with benign lesions or ALH/LCIS (Med 60%, range 20-90%)(p=0.012). No other histologic features on CNB (e.g. degree of sclerosis, apocrine metaplasia or hyperplasia) were predictive of ADH or DCIS at excision. Median Topo% was 1.2 for cases with ADH or DCIS at excision vs 3.6 for those without (p=0.048). Distribution of p63 and CK5/6 immunostaining was similar amongst CNB cases regardless of the excision diagnosis. Imaging data (target size, site, biopsy method, gauge, core #) were non-contributory.

Conclusions: A significant proportion of patients with PapWA on CNB had either DCIS (8%) or ADH (8%) at excision. Apart from the percentage of CNB involved by PapWA, neither CNB pathologic features nor imaging data were helpful in predicting the probability of carcinoma or atypia at excision. Our findings suggest that surgical excision remains prudent for all patients with PapWA on CNB due to the risk of more significant pathology occurring in the targeted lesion.

157 Should Intraductal Papilloma Diagnosed on Core Needle Biopsy Be Excised?

S Jaffer, CS Nagi, IJ Bleiweiss. The Mount Sinai Medical Center, New York, NY.

Background: While it is known that intraductal papillomas with atypia or malignancy (IDPAM) diagnosed on core needle biopsy (CNB) must be excised, the management of intraductal papillomas without atypia or malignancy (IDP) remains controversial. Based on the few small existing series that suggest a small but definite chance of atypia or malignancy of IDP, we recommend excision of all IDP on CNB. The purpose of our study was to evaluate the pre cancerous potential of IDP by evaluating our follow up data of excisions of IDP diagnosed on CNB.

Design: Using the computerized pathology files from 1/2000 to 8/2004, we identified 200 cases of IDP diagnosed on CNB (ultrasound guided=136, mammotome=64). Information regarding excision was available in 99 cases. Four patients refused further surgery and remain clinically stable. Follow up information is pending on the remaining patients.Histologic sections of both the CNB and excision specimens were reviewed in the 99 cases to confirm the CNB diagnosis of IDP, correlate with the excision diagnosis and identify biopsy site changes.

Results: The age of the patients ranged from 25 to 82 years (mean=55.5). Histologic review of the excision specimens revealed biopsy site changes in all cases. The diagnoses were as follows: IDP=65 cases (68.5%), no residual IDP=16 (16.8%) and IDPAM=14 (14.7%). The IDPAM ranged from atypical duct hyperplasia (ADH)=6 cases, intraductal carcinoma only (DCIS)=5, and DCIS with invasive carcinoma (IC)=3. All cases of ADH were microscopic, involved IDP and were present at the previous biopsy site. All cases of IC were adjacent to but not at the previous biopsy site which contained DCIS involving IDP. All cases of DCIS were present at the previous biopsy site and showed a spectrum of histologic changes ranging from florid to atypical duct hyperplasia to DCIS, all involving IDP. Radiologic pathologic correlation in the IDPAM indicated that in all the IC, the radiologic findings were suspicious for malignancy, such that excision would have been performed despite the absence of a diagnosis of IDPAM. This was also true in one case of DCIS which had pleomorphic calcifications.

Conclusions: This large series of IDP on CNB indicates that while most IDP diagnoses on excision remained the same, approximately 15% were upgraded to either atypia (6.3%) or malignancy (8.4%), most likely due to sampling error. The close proximity of atypia or malignancy to the IDP suggests the pre cancerous potential of these lesions. Close radiologic pathologic correlation is important in the evaluation of these lesions.

158 Prediction of Non-Sentinel Lymph Node Metastases in Sentinel Node Positive Invasive Breast Carcinomas

U Kapur, TC Rubinas, S Gabrum, JM Sinacore, PB Rajan, K Yao. Loyola University Medical Center, Maywood, IL.

Background: The incidence of non-sentinel (NSN) lymph node metastases in patients with a tumor positive sentinel lymph node (SN) varies greatly from 20-70%. It appears that a large number of patients with a tumor positive SN do not necessarily need a complete axillary dissection. Unfortunately, we do not have any reliable predictive factors to select which patients with a tumor positive SN will have tumor involved NSN without removing the axillary nodes. The aim of this study is to correlate the primary tumor morphological features and SN metastases with NSN status and to find out the factors that are predictive of NSN metastases.

Design: We examined the pathology slides from 64 invasive breast carcinoma patients with tumor positive SN who had undergone a complete axillary dissection. We reviewed the slides for tumor size, histological type, histological grade (Modified Bloom-Richardson), lymphovascular invasion, number of tumor positive SN, size of SN metastases, NSN status, estrogen and progesterone receptor and HER-2/neu oncoprotein expression. Fisher's exact test and students t test were used for statistical analysis.

Results: Out of 64 invasive breast carcinoma patients with positive SN, 19 had NSN metastases (29.1%). Primary tumor size ($p< .002$) and size of SN metastases ($P< .03$) were significantly associated with NSN metastases. Primary tumor size of patients with NSN metastases ranged from 1.5 to 11cm with a mean of 2.1cm. Size of SN metastatic tumor size varied from 0.2 cm to 2.4 cm with a mean of 1.0 cm. The association between tumor type, tumor grade, lympho-vascular invasion, estrogen and progesterone receptor and Her2/neu oncoprotein status were not statistically significant ($P> .05$).

Conclusions: Primary tumor size ($P< .002$) and size of SN metastases ($P< .03$) were significantly associated with positive NSN status in this analysis. We conclude that infiltrating mammary carcinomas with a mean primary tumor size of 2.1cm and mean SN metastatic tumor size of 1.0 cm have a high probability of NSN metastases

159 Pathology of the Borderline HER-2/*neu* Breast Carcinoma

JL Killeen, A Ortega-Lopez, JB Fu. Kapiolani Medical Center for Women and Children, Honolulu, HI.

Background: The significance of HER-2 results obtained by immunohistochemical analyses which are neither negative or strongly positive is controversial. Many studies show that the incidence of FISH positivity in these tumors is small and the implication is that these borderline results more likely represent laboratory misclassification.

Design: HER-2/*neu* status was determined by image analysis of IHC stained sections in 519 consecutive cases of invasive breast carcinoma utilizing a method shown to correlate closely with HER-2 analysis by FISH. All cases in the borderline category were reflex tested by FISH to determine HER-2 gene status. Morphologic data recorded included overall tumor grade, grading components (nuclear grade, tubule formation, mitotic activity) and prognostic markers determined by image analysis including estrogen receptor, progesterone receptor and Ki-67.

Results: HER-2 was negative for overexpression in 372 (71.7%), positive for overexpression in 75 (14.4%) and borderline in 72 (13.9%). FISH was successfully performed on 71 of the 72 borderline cases. Of the 71 borderline cases tested by FISH, 14 (19.7%) were positive for amplification. Analysis of morphologic and molecular markers showed that the borderline group has a phenotype similar to the HER-2 negative group and significantly different from the HER-2 positive group for ER and PR. The borderline set was then divided into FISH-negative and FISH-positive groups. Borderline HER-2/FISH-negative tumors were statistically similar to HER-2 negative tumors for all parameters. Borderline HER-2/FISH-positive tumors, however, showed a profile distinct from HER-2 positive tumors with statistically significant differences

for ER and PR compared to the HER-2 positive group. When analyzed as continuous variables, both ER and PR showed significantly higher mean levels in borderline HER-2/FISH-positive tumors compared to clear HER-2 positive tumors.
Conclusions: The morphologic and molecular features of breast carcinomas showing borderline HER-2 expression suggest that these are a unique group of lesions. While borderline tumors which are FISH-negative have a phenotype similar to HER-2 negative tumors, borderline tumors that are FISH-positive do not express a phenotype that matches the phenotype of overtly HER-2 positive tumors. These tumors do not simply represent examples of laboratory imprecision but rather identify a truely borderline group. These findings are intriguing in light of the recent evidence highlighting the interaction between HER-2 and hormone receptors.

160 Comparative Analysis of Her2/Neu Status with Histopathologic Parameters and Hormone Receptor (HR) Status in Breast Carcinomas (Ca)

JW Kim, MJ Kim, J Choi, GY Gong, JY Ro. University of Ulsan College of Medicine, Seoul, Korea.
Background: The Her2/neu oncogene is amplified in 20-30% of invasive breast Ca and known to have prognostic and predictive values. Overexpression of Her2/neu is generally known to be associated with higher tumor grade and decreased expression of HRs, however, frequency of Her2/neu expression according to HR status and histologic subtype is not well established.
Design: Immunohistochemical (IHC) stainings for estrogen receptor (ER), progesterone receptor (PR), p53, and Her2/neu were performed on formalin-fixed, paraffin embedded tissue blocks of 439 invasive breast Ca. ER, PR, and p53 were graded as 0, 1^+, 2^+, and 3^+ and interpreted as positive if more than 10% of tumor cells show nuclear staining. The IHC results for Her2/neu were scored on a 0 to 3^+ scale according to the standard criteria. FISH for Her2/neu was performed on 52 cases with IHC score 2^+. Her2/neu positivity (+) was defined as tumors with IHC score 2^+ having FISH amplification or IHC score 3^+.
Results: Of 439 invasive breast Cas, 363 cases were invasive ductal Ca, not otherwise specified (NOS) and 76 cases were special variants. Overall Her2/neu + was 23.0% (101 of 439 cases) with 90 cases of IHC score 3^+ and 11 cases detected by FISH amplification. Of 52 cases with IHC score 2^+, 11 were of FISH positive cases (21.2%). According to histologic subtypes, none of invasive lobular Ca (classic;8, pleomorphic;1), metaplastic Ca (4), tubular Ca (5), tubulolobular Ca (2), and atypical medullary Ca (3) showed Her2/neu +, while one each of mucinous (15) and cribriform Cas (3) and six out of 35 micropapillary Cas showed Her2/neu +. Except for micropapillary Ca, variant Cas showed a significantly low Her2/neu + (2 of 41 cases, 4.9%) compared with invasive ductal Ca, NOS (93 of 363 cases, 25.6%) ($p<0.05$). Her2/neu + was associated with higher nuclear and histologic grades and p53 + ($p<0.05$). Although Her2/neu was inversely correlated with ER or PR + ($p<0.05$), considerable proportion of Her2/neu positive tumors coexpressed ER (40.6%) and PR (36.6%). Between Her2/neu$^+$/ER$^+$/PR$^+$ and Her2/neu$^+$/ER$^-$/PR$^-$ groups, only tumor grade was statistically different ($p<0.01$).
Conclusions: Her2/neu overexpression was intimately associated with histologic subtypes and tumor grade of breast Ca. Breast Cas, NOS more expressed Her2/neu than special variant breast Cas. Although Her2/neu + was inversely correlated with ER and PR status, more than one third of tumors showed coexpression of Her2/neu with ER and/or PR.

161 CISH with a Centromeric Probe for Chromosome 17 Can Be as Accurate as FISH in Detecting Polysomy in Breast Carcinomas

E Kostopoulou, M Ioannou, M Nakou, G Kalodimos, M Netsika, G Koukoulis. University of Thessaly, Larissa, Greece.
Background: Chromogenic in situ hybridization (CISH) compared to fluorescence in situ hybridization (FISH) offers superior morphologic correlation, eliminates the need for special microscopy and facilitates data storage and re-evaluation, but until now it has not replaced FISH as the gold standard for the detection of c-erbB2 amplification in breast carcinomas. In previous attempts of routine application of CISH, two major obstacles were the difficulty in obtaining discernible signals with a probe for chromosome 17 or the unavailability of that probe.
Design: The number of signals of chromosome 17 was determined in 60 randomly selected neoplastic cell nuclei in each one of 92 cases of infiltrating ductal breast carcinoma by applying centromeric probes, initially using FISH and subsequently CISH. FISH was performed using a PathVysion kit (Vysis, Downers Grove IL) according to the instructions provided by the manufacturer. CISH was also performed according to the manufacturer's instructions (Zymed, San Francisco Ca) with few modifications. The CISH slides were reviewed blindly and independently by 2 observers without information on the previous findings by FISH or immunohistochemistry and the results were compared following the completion of the entire review.
Results: The CISH signals were easily detectable and well defined. However, their precice quantification required the use of a 60X objective lens without oil immersion. Polysomy was present in 36/92 (39%) cases. In 30/92 cases polysomy was identifiable similarly by both methods. Discrepancies were noted in six cases (6.5%). Those included 3 cases with polysomy found only by FISH and 3 cases with polysomy detected only by CISH. In the other 56 cases, mostly with disomy, the results were identical.
Conclusions: The detection of the signals of chromosome 17 and especially the identification of polysomy using CISH was as accurate as that by FISH and it should not be a problem hindering the wider application of CISH in the evaluation of c-erbB2 amplification in breast carcinomas. It may even be superior to FISH in cases with attenuated cords of infiltrating neoplastic cells admixed with inflammatory elements or in cases with minute foci of invasive carcinoma. There are however very few cases with weak, barely detectable signals, where FISH shows brighter signals and superior reliability.

162 Evaluation of HER-2/*neu* Status in Breast Carcinomas: A Combined Approach Including FISH, CISH, Real Time QRT PCR and Immunohistochemistry

E Kostopoulou, D Kaisaridou, M Ioannou, N Vladica, M Nakou, G Kalodimos, M Netsika, G Koukoulis. University of Thessaly, Larissa, Greece.
Background: Many studies have portrayed a static view of the HER-2/*neu* status in breast carcinomas either by immunostaining receptor epitopes or by estimating the amplification (Amplf) of the pertinent gene. A more panoramic or dynamic view including mRNA computation with real time QRT PCR has been attempted infrequently and with various results questioning the choice of the gold standard test.
Design: One hundred and eighteen selected cases of ductal infiltrating breast carcinomas were examined by fluorescence in-situ hybridization (FISH, PathVysion, Vysis) and/or chromogenic in-situ hybridization (CISH, HER2DNA probe, chromosome 17 centromeric probe, ZYMED). Real time QRT-PCR was performed in 25/118 cases (Robo-Gene HER2/NEU and GAPDH cDNA quantification Modules, Roboscreen, Rotor-Gene 2000). In the latter method, every tumor sample was obtained fresh and was compared to a normal breast sample from the same patient. Overexpression was characterized as strong or moderate based on the HER2/GAPDH ratios of tumor/normal samples. Immunohistochemistry (IHC) was performed using CB11 (Biogenex).
Results:

Evaluation of HER-2/neu status in breast carcinomas

IHC	Amplf FISH and/or CISH	QRT PCR overexpression	Polysomy chr. 17
3+,31	28*	4/4*	13
2+,32	5**	6/12**	19
1+,41***	2*	3/6*	11
0,14	0	0/3	0
total : 118	35	13/25	43

* In the 3 cases with 3+ IHC and no amplification, and in the 2 cases with 1+ IHC and amplification, there were no samples for QRT PCR** In 3/5 cases with amplification, there were samples for PCR and showed overexpression. *** All 4 studied cases with 3+ IHC showed strong overexpression by PCR.

In 10 cases with 1+ or 2+ IHC and moderate overexpression, no amplification was found whereas polysomy 17 was noted in 6/10.
Conclusions: Detection of HER-2/*neu* amplification by FISH or CISH will continue to be the favored method for further evaluation of cases with 2+ and of some cases with 1+ immunoreactivity. However our findings indicate, in keeping with recent reports, the emergence of a small, albeit not negligible, group of cases characterized by limited immunoreactivity, lack of gene amplification and moderate mRNA overexpression. The clinical significance of these findings is not known and its clarification would require implementation of methodology similar to that of our study.

163 Stromal Cells of Some Pre-Invasive Breast Lesions Exhibit Biological Attributes of Stromal Cells Associated with Breast Cancers

S Kraeft, L Collins, J Sneddon, P Brown, M van de Rijn, S Schnitt. Beth Israel Deaconess Med Ctr, Boston, MA; Stanford University, Stanford, CA.
Background: The importance of stromal-epithelial interactions in established breast cancers is well recognized, but the role of stromal cells in early stages of breast cancer initiation and progression is less well defined. It is possible that the stromal cells of some pre-invasive breast lesions already exhibit biological characterstics of stromal cells of invasive breast cancers, and this could create conditions (via paracrine signaling) favoring cancer development in women with pre-invasive lesions.
Design: We constructed tissue microarrays (TMAs) consisting of quadruplicate cores of stroma from non-proliferative lesions (NP), usual ductal hyperplasia (UDH), sclerosing adenosis, atypical ductal hyperplasia (ADH), non-high grade ductal carcinoma in situ (DCIS), high grade (HG) DCIS (n=20 each) and radial scars (RS, n=13). Twenty cases each of normal stroma, stroma from infiltrating ductal carcinomas, and biopsy sites were included for comparison. TMA sections were immunostained using antibodies to proteins expressed in stromal cells of invasive breast cancers including smooth muscle actin (SMA) and syndecan-1 (CD138), and to 5 proteins expressed by fibroblasts after serum exposure that have been implicated in wound healing and tumor progression (serum response proteins Id3, nme1, coro1c, loxl2 and plod2) (Chang, PLoS Biology, 2004).
Results: Among the pre-invasive lesions studied, SMA was expressed by stromal cells in 90% of HG-DCIS, 77% of RS, 45% of non-high grade DCIS, and 15% each of UDH and ADH. One or more of the serum response proteins was expressed by stromal cells in 30% of HG-DCIS, 30% of RS, 25% of ADH, and 20% of UDH. Among the serum response proteins evaluated, Id3 (an inhibitor of DNA binding) was the most often expressed in the pre-invasive lesions. Syndecan-1, expressed in stromal cells of 25% of invasive cancers, was only infrequently noted in stromal cells of pre-invasive lesions (seen in 10% of HG-DCIS and 10% of UDH only).
Conclusions: Stromal cells associated with some pre-invasive breast lesions already show expression of several proteins characteristic of those expressed by stromal cells of invasive breast cancers, including SMA and several serum response proteins. Whether the expression of these markers in stromal cells identifies a subset of patients with pre-invasive lesions at increased risk for progression to invasive breast cancer is currently being evaluated in cohorts of patients for whom clinical follow-up is available.

164 The Detection of Isolated Tumor Cells in Bone Marrow Comparing Brightfield and Multi Color Fluorescent Microscopy

DN Krag, R Kusminsky, E Manna, AB Ambaye, D Weaver, SP Harlow, M Covelli, MA Stanley, L McCahill, F Ittleman, B Leavitt, M Krag. University of Vermont, Burlington, VT; The CAMC Institute, Charleston, WV.
Background: The detection of isolated tumor cells (ITCs) in bone marrow by immunocytochemistry has been reported to predict progression of early stage breast cancer. The most common staining procedure utilizes bright-field immunocytochemistry (BFI) with cytokeratin antibodies to label ITCs. However, this

method may result in false positive staining events which would influence detection and prognostic significance. Our goal was to use multi-color fluorescence immunocyotchemistry (MFI) to develop a more specific assay for detecting ITCs in marrow samples from breast cancer patients.
Design: We compared BFI and MFI on bone marrow aspirates from breast cancer patients, bone marrow from healthy donors, and healthy donor blood spiked with cancer cells. The primary target for ITC detection was cytokeratin for both methods. MFI used an additional set of antibodies to label hematopoietic cells (HC).
Results: The detection rate of cytokeratin positive (CK+) events in breast cancer patient bone marrow aspirates was 14/26 (54%) for BFI and 16/26 (61 %) for MFI. However, with MFI 12/16 CK+ cases were stained with HC markers and identified as false positive cases. A surprisingly high CK+ event rate was observed in donor blood and marrow. In all donor samples, CK+ events were readily identified as HCs by MFI. Detection sensitivity of spiked cancer cells in donor blood was similar for both methods.
Conclusions: There is a high frequency of CK+ events in blood and marrow and importantly this is observed both in cancer and non cancer patients. MFI with multiple HC markers allows discrimination with higher confidence than BFI between CK+ cells of hematopoietic and non-hematopoietic origin.

165 Pathological Changes Following Adenoviral p53 Gene(Advexin) Therapy for Locally Advanced Breast Tumor

S Krishnamurthy, LT Guerra, V Valero, M Cristofanilli. M. D. Anderson Cancer Center, Houston, TX.
Background: There is as yet no report of the efficacy and pathological changes in breast tissue following p53 gene therapy. We report here the findings in locally advanced breast cancers (LABC) following intratumoral injection of Advexin which contains the wild type p53 transgene tagged to an adenovirus vector.
Design: Twelve patients with LABC were treated with intratumoral injections of Advexin (days 1,and 2 of each cycle) and chemotherapy with docetaxel and doxorubicin. Core biopsy was performed before therapy and samples were evaluated for ER/PR, HER2/neu, and p53 mutation status. Fine-needle aspiration biopsy (FNAB) was performed on day 1 before therapy and on days 3, 4, and 21; samples were evaluated for p53 mRNA levels by RT-PCR. After 6 cycles of Advexin injections and chemotherapy, patients underwent mastectomy and complete axillary dissection. Immunostaining was performed using antibodies against CD3, CD20, CD4, and CD8 to characterize the lymphocytic infiltration in the tumor.
Results: Median size of the breast tumors before therapy was 8.0 cm (range, 5.0 - 11.0 cm). Tumors were positive for ER in 6 cases, PR in 1, and HER2/neu in 4. Eight patients had missense mutations in exon 5-8, intron 4. Sequential increases in p53 mRNA levels were found in 6 (86%) of 7 patients in whom RNA was available from all FNABs. Mean residual tumor size was 1.78 cm (range, 0.1 - 6.0 cm) and large areas of fibrosis were present in all cases. There was no correlation between base line p53 mutation and residual disease or increase in p53 mRNA. All except one patient had metastasis to axillary lymph nodes (mean – 6/18). The most notable pathologic finding was moderate to marked lymphocytic infiltration comprising predominantly of CD8 T cells in all the cases. Increased CD8 T cells were associated with less residual disease (p=0.03).
Conclusions: 1) The presence of significantly increased cytotoxic CD8 T cell infiltration in and around the invasive tumor is a consistent finding following Advexin therapy.
2) The gene transfer efficacy of Advexin therapy is demonstrated by sequential increases in p53 mRNA levels in 86% of patients in this study. 3) Induction of apoptosis of breast cancer cells following Advexin therapy is probably related to increases in p53 mRNA levels and cytotoxic T cells. 4) Advexin therapy in combination with chemotherapy had less effect on locoregional metastasis in comparison to the primary breast tumor.

166 Total Embedded Axillary Tissue Does Not Affect N Stage of Breast Cancer

Y Kwon, EK Hong, JS Ro, ES Lee, JY Ro. National Cancer Center, Goyang, Gyeonggi-do, Republic of Korea; Asan Medical Center, Seoul, Republic of Korea.
Background: Number of metastatic axillary lymph nodes (LNs) determines N substage of breast cancer. The aim of this study is to find out whether total submission method (TSM) affects N stage of breast cancer in comparison with the routine manual dissection method (MDM).
Design: 82 mastectomy/lumpectomy specimens with axillary LN dissection at National Cancer Center and Asan Medical Center from May to Aug 2004 were selected. Initially the axillary LNs were manually dissected from the axillary contents (MDM). The entire remaining axillary tissue was totally embedded for additional LN identification (TSM).
Results: Mean number of lymph node obtained by initial MDM was 17.4 (range 4-33). Number of additional paraffin blocks examined by TSM ranged from 1-22 with a mean of 10.2 blocks, and mean number of lymph node harvested was 5.9 (range 0-21: sum of LNs, 481). Most of additional LNs were small, less than 2mm. The initial MDM identified LN metastases in 43 cases (52.4%). Among these node positive cases, additional LN metastases (range 1-5) were identified in 9 cases by TSM. Of these 9 cases, N stage was changed in only 2 cases [N1a → N2a(IIB → IIIA), N2a → N3a(IIIA → IIIC)] and unchanged in the remaining 7 cases. Among the initial 39 node negative cases, none showed metastatic LN by TSM.
Conclusions: TSM for further LN isolation did not result in statistically significant change in N stage of breast cancer when compared with a standard MDM. Moreover, the routine MDM from axillary fat contents accurately reflected node negative status. Therefore, considering labor intensity and cost, TSM for further LN isolation added little benefit and is not advised for routine practice.

167 Immunohistochemical Analysis of HER-2 in Tumors Showing an Increased Number of HER-2 Genes but a HER-2:CEP-17 Ratio of Less Than 2

AJ Kyshtoobayeva, KJ Bloom. ChromaVision Medical Systems, Irvine, CA.
Background: There is generally good concordance between tumors showing 3+ over-expression of HER-2 by immunohistochemistry (IHC) and amplification of the HER-2 gene. The aim of this study was to look at the immunohistochemical expression of HER-2 in tumors with an increased average number of HER-2 gene per cell, but a HER-2:CEP-17 ratio of less than 2.0.
Design: 9546 consecutive breast cancers assessed for HER-2 gene amplification at US Labs were reviewed. FISH was performed using the PathVysion assay (Vysis, Downer's Grove, IL). Twenty cells were assessed and the number of HER-2 and CEP-17 signals were counted in each cell, up to twenty signals each. Tumors showing a HER-2:CEP-17 ratio of less than 2.0 but an average HER-2 count of greater than 6 were evaluated by IHC using the HercepTest kit (DakoCytomation, Carpinteria, CA). IHC assessment was performed manually by a single experienced pathologist.
Results: 7383 (77.3%) of the reviewed samples had a HER-2:CEP-17 ratio of less than 2.0. 948 tumors (12.8%) showed an average HER-2 count greater than or equal to 4 and 130 of these tumors (1.8%) showed an average HER-2 signal count of greater than 6. Forty-three tumors were available for IHC analysis. Six tumors (14%) showed 3+ expression by IHC, 12 (27.9%) showed 2+ expression, 11 (25.6%) showed 1+ expression and 14 (32.6%) showed 0+ expression. Only tumors with an average HER-2 count of greater than 16.5 showed 3+ expression by IHC. Three other tumors showed an average HER-2 count of greater than 16.5 and all three showed 2+ expression.
Conclusions: Of the 7383 tumor assessed as non-amplified, 1.8% had an average HER-2 count of greater than 6 copies per cell. In an analysis of a subset of these cases only 14% showed 3+ over-expression by IHC and all had average HER-2 counts of greater than 16.5. However, only 67% of tumors with an average HER-2 count of greater than 16.5 and a HER-2:CEP-17 ratio of less than 2 showed 3+ over-expression. While aneusomy of chromosome 17 does occur, it does not appear to be a significant cause of 3+ over-expression.

168 Molecular Diagnosis of Breast Cancer Therapeutic Biomarkers Using Oligonucleotide Arrays

P Lal, M Donaton, D Giri, B Chen, WL Gerald. University of Pennsylvania, Philadelphia, PA; Memorial Sloan-Kettering Cancer Center, New York, NY; Brown University, Providence, RI.
Background: High through put technology such as DNA microarrays allows analysis of large numbers of genes in a single experiment. This technology has the potential to not only identify new diagnostic markers but also provide an efficient means to evaluate many clinically relevant biomarkers in a single diagnostic procedure. To test the efficacy of DNA chip technology as a potential diagnostic tool we compared mRNA expression of several well established diagnostic markers in breast cancer(ER, PR and HER2) based on oligonucleotide arrays, to results using FDA approved immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) tests.
Design: Expression analysis of 115 breast cancer samples was performed on Affymetrix U133 human gene oligonucleotide arrays. **ER, PR and Her 2/neu IHC and Her 2/neu FISH** was performed on the same samples using paraffin-embedded multi-tissue block with diagnostic kits (Ventana for ER and PR, Dako for Her 2/neu IHC, and Vysis for Her 2/neu FISH) and interpreted by clinical parameters. **Statistical analysis for** sensitivity and specificity was performed using the Receiver Operator Characteristics analysis [ROC, statistical package, SPSS (SPSS Inc. Chicago IL)].
Results: Despite the fact that different molecular components are being queried, there was a strong correlation between results using oligonucleotide arrays for measurement of RNA levels and FDA approved diagnostic assays for protein levels or gene copy number (table 1). The status of ER was highly reliably predicted and mRNA expression of Her2/neu correlated better with FISH than IHC.
Conclusions: It is likely that DNA microarray technology can be used to evaluate the status of clinically relevant markers in breast cancer. Eventual utility will be dependent on prospective studies to determine the correlation between various diagnostic tests and therapeutic response
Table 1: ROC curve analysis

	Area under the curve	Std Error	95% CI Lower Bound	95% CI Upper Bound
Prognostic Marker				
ER probe vs ER IHC	0.977	0.013	0.951	1.002
Her 2/neu probe vs IHC	0.765	0.089	0.59	0.939
Her 2/neu probe vs FISH	0.886	0.08	0.73	1.05
PR probe(a) vs IHC	0.876	0.38	0.802	0.95
PR probe(b) vs IHC	0.563	0.6	0.446	0.681

ROC= Receiver operator Characteristics; CI=confidence interval;

169 Benefits and Pitfalls of Tumor Size and/or Volume Determination on Core Needle Biopsy in Invasive Breast Cancer: A Year of Experience from a Community Hospital

JF Lara, RG Abellar, NV Singh. Saint Barnabas Medical Center, Livingston, NJ.
Background: Tumor size is vital to the breast cancer report. It determines prognostic significance and the need for adjuvant therapy. Core needle biopsy (CNB) is increasingly used. Stereotatic localization and ultrasound guided biopsies can yield multiple cores and completely remove small neoplasms making assessment of tumor size impossible (pTx). Radiologic study overestimates size by up to 30%. There is a need to assess tumor size determination on CNB.
Design: A one year retrospective study was initiated to determine accuracy of tumor size and volume on CNB. Breast cancers with CNB and resection were identified. Tumor volume was estimated as a percentage of the CNB. Size was based on the longest

measurement of tumor on the slide and tumor to the tips of the cores. Macro and microscopic tumor size on resection was determined and compared with the CNB. DCIS foci were excluded. Fragmented tumor and biopsy angle were considered.
Results: 72 cancers (61 ductal, 11 lobular) were analyzed. Tumor volume on CNB's ranged from 10 to 90%; size ranged from 0.2 to 2.3 cm. There was residual tumor in 66 of 72 cases (8% pTx) and size discordance in 63 (95%). Tumor size was greater in the resections of 53 cases (84%) , but in 10 cases, CNB was the larger size (16%). 3 cases were equal. When stage (TNM) was assessed, 26 cases (39%) changed by one stage (ex. T1c to T2), 22 (33%) by 2 or more (ex. T1b to T2) and 18 (27%) did not change. Tumor measurement went from less than 1.0 cm to over 1.0 cm. in 27 cases (40%) but the core was larger in 8 of those cases (30%). The CNB had the definitive measurement in 16 of 72 cases (22%). Many of the CNB's were fragmented and tangential sections were observed suggesting biopsy of the tumor periphery. Amount of DCIS and tumor volume was not a factor in determining tumor size. Radiographic data was not additive.
Conclusions: Tumor size and volume cannot be accurately determined on CNB in most cases, resulting in upstaging at resection (72%). CNB did represent the definitive tumor size in 22% of cases in this study, especially small tumors either completely or almost completely removed by CNB. Determining tumor size on CNB is limited by technique and tumor friability. Based on these results it is important to attempt to determine tumor size from CNB's when possible especially in small tumors that may have been totally removed. But it is prudent to underscore the limitation of the measurement and urge correlation with the resection whenever possible.

170 Expression of Cyclooxygenase-2 in Breast Carcinogenesis and Its Relation to p53 and HER-2/neu Protein Expression in Invasive Ductal Carcinoma

JS Lee, HJ Kim, JH Lee, JH Nam, C Choi, MC Lee, CS Park, SW Juhng, JH Yoon, KW Min. Chonnam National University Medical School, Gwangju, Republic of Korea; Deaconess Hospital, Oklahoma, OK.
Background: Cyclooxygenase-2 (COX-2) is over-expressed in malignant tumors including breast cancers, but little is known about the contributions of COX-2 in breast carcinogenesis. Recent studies suggest a possible role of p53 and HER-2/neu gene in controlling COX-2 regulation. The purpose of this study was to evaluate COX-2 expression in the successive steps of breast carcinogenesis and to determine its correlation with p53 and HER-2/neu expression in invasive ductal carcinomas of the breast.
Design: Immunohistochemical staining with anti-COX-2 antibody was performed in normal breast tissue (n=15), florid ductal hyperplasia (n=15), ductal carcinoma in situ (n=30), and invasive ductal carcinoma, not otherwise specified (n=99). Expression of COX-2 in invasive ductal carcinoma was correlated with p53 and HER-2/neu protein expression as well as clinicopathologic features.
Results: COX-2 expression progressively increased along the continuum from normal breast epithelium to invasive ductal carcinoma ($p<0.001$). Increased COX-2 expression in invasive ductal carcinoma did not significantly correlate with age, tumor size, tumor grade, lymph node status, lymphovascular invasion, or TNM stage. Increased COX-2 expression was significantly associated with p53 and HER-2/neu protein expression ($p< 0.05$ and $p<0.001$). On multivariate analysis, however, only TNM stage and increased COX-2 expression correlated with survival.
Conclusions: Our results suggest that increased expression of COX-2 may be involved in the carcinogenesis of the breast and may be an independent prognostic indicator in patients with invasive ductal carcinoma. p53 and HER-2/neu are likely to be involved in the regulation of COX-2 expression in invasive ductal carcinoma of the breast.

171 Apocrine Metaplasia and the Development of ER-Negative Breast Cancer

S Lee, SK Mohsin, H Weiss, D Medina, DC Allred. Baylor College of Medicine, Houston, TX.
Background: Benign breast epithelium commonly undergoes a transition to apocrine-like cells (termed "apocrine metaplasia"), which is associated with a near-total loss of ER expression. It is not until the late stage of DCIS during breast cancer evolution that a significant population of ER-negative precursors emerges, and it is likely that progression of a subset of ER-negative DCIS accounts for the majority of ER-negative invasive disease. We have noted a continuum of apocrine features in DCIS ranging from none to extensive, and wondered whether it was associated with ER status. It makes sense that malignant cells might continue to demonstrate some of the same behaviors as their benign ancestors, such as becoming ER-negative through apocrine metaplasia.
Design: To address this issue in a preliminary manner, we recently performed a blinded assessment of histological grade, ER expression, and the extent of apocrine histological features in a series of consecutive DCIS (n = 172), and looked for correlations between them. A modification of the Scarff-Bloom-Richardson method was used to assess grade. Standard immunohistochemical techniques were used to measure ER status (Allred Score ranging from 0 to 8). An apocrine score was developed to estimate the degree of histological apocrine features (0 = none; 1 = mild; 2 = moderate; 3 = extensive).
Results:

Correlations of Apocrine Scores, Histological Grade, and ER Scores in DCIS (n=172)

	Apo Score 0	Apo Score 1	Apo Score 2	Apo Score 3
Grade 1	78% (n=46)	19% (n=11)	3% (n=2)	0% (n=0)
Grade 2	46% (n=21)	37% (n=17)	15% (n=7)	2% (n=1)
Grade 3	54% (n=36)	42% (n=28)	4% (n=3)	0% (n=0)
Mean ER Score:	4.99	3.80	2.58	0.00

As apocrine features increased, there was a corresponding increase in histological grade (overall p=0.002), and a corresponding decrease in ER expression (overall p=0.001). Thirty one percent of all cases (53/172) were ER negative. Among the ER negative cases, 53% showed some evidence of apocrine differentiation (apocrine scores > 0), and 15% showed a high degree of apocrine differentiation (scores = 2 or 3).
Conclusions: These observations are consistent with the hypothesis that a mechanism associated with apocrine metaplasia may be involved in the development of a substantial proportion of ER-negative breast cancer. Microarray experiments are ongoing to identify the changes in gene expression associated with apocrine metaplasia and the accompanying loss of ER.

172 Analysis of Cancer Risk among Patients with Papillary Lesions of the Breast

JT Lewis, RA Vierkant, SD Maloney, LC Hartmann, DW Visscher. Mayo Clinic, Rochester, MN.
Background: Papillomas are relatively common breast lesions. Although most are single and histologically bland, they may be multiple and demonstrate varying degrees of atypia. The risk of breast carcinoma development in patients with benign papillary breast lesions is incompletely defined.
Design: Papillary breast lesions were identified in a histopathologically-defined benign breast disease cohort of 8872 patients biopsied between 1967-1991. Cases were subclassified into four groups: single papilloma without atypia, single papilloma with atypia, multiple (>3) papillomas without atypia, and multiple papillomas with atypia. Using Cox proportional hazards regression, the risk of cancer development among these groups was compared to patients with other forms of proliferative breast disease (with or without atypia) and patients with non-proliferative breast changes.
Results: Of the 368 patients diagnosed with a single papilloma without atypia, 35 (10%) developed carcinoma. Eleven (22%) of the 49 women with a single papilloma with atypia subsequently developed carcinoma. Forty-one patients were diagnosed with multiple papillomas without atypia, and six (15%) developed carcinoma. Twelve cases of multiple papillomas with atypia were identified, and 4 (33%) of these developed carcinoma. The relative risk of cancer development is presented in Table 1.
Conclusions: We conclude that the diagnosis of a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other non-atypical forms of proliferative breast disease. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias.

Incident Breast Cancer Relative Risk

Diagnosis (N)	Person Years Followup	Relative Risk (95% CI)
Non-Proliferative (5934)	91129	1.00
Proliferative without Atypia (2211)	32895	1.60 (1.35, 1.90)
Proliferative with Atypia (257)	3127	3.59 (2.63, 4.92)
Single Papilloma without Atypia (368)	4979	1.82 (1.28, 2.58)
Single Papilloma with Atypia (49)	577	4.88 (2.67, 8.92)
Multiple Papillomas without Atypia (41)	592	2.81 (1.25, 6.31)
Multiple Papillomas with Atypia (12)	115	8.66 (3.22, 23.31)

Relative risks were calculated using a Cox proportional hazards regression analysis. Results are adjusted for age.

173 Chromogenic *In Situ* Hybridization (CISH), a Helpful Tool for the Pathologist To Identify HER2/NEU Amplification in Breast Carcinomas. Comparative Study with IHC and FISH

EML Li Ning, R Ronchetti, CA Torres-Cabala, LS Teller, MJ Merino. NCI/NIH, Bethesda, MD.
Background: The FDA-approved methods to evaluate HER2 status in breast cancer analyze HER2 protein expression through immunohistochemistry (IHC) or HER2 gene amplification through fluorescence *in situ* hybridization (FISH). FISH is an expensive technique not available in most laboratories, but it remains the gold standard because it has a better correlation with prognosis and response to therapy than IHC. Discrepancies between IHC and FISH results have been attributed to a number of factors, including chromosome (Ch) 17 ploidy, technical variations in IHC, and the type of tumor examined. CISH is a new method that can be used by the surgical pathologist, to evaluate gene amplification at a lower cost than FISH using a visualization technique similar to IHC.
Design: The purpose of this study was to evaluate the utility of CISH to determine HER2 status in breast carcinoma. To avoid misinterpretations due to polysomy, CISH for Ch 17 was performed on separated slides for all the cases. A total of 55 cases of paraffin-embedded invasive breast carcinomas were included. They consisted of the following histologic tumor types: lobular (n=5), ductal (n=42), pleomorphic lobular (n=2) and micropapillary (n=6). FISH and IHC were also performed and the results compared.
Results: CISH showed a 96.9% agreement with FISH. Aneuploidy of Ch 17 by CISH was useful to discriminate between true amplification and pseudoamplification, especially when there was a low increase in the number of HER2 gene copies (6-10 signals per nucleus) allowing to reclassify 4/6 of these cases as non-amplified. The overall agreement with IHC for 0/1+, 2+ and 3+ scored cases was 100%, 17.86% and 84.61% respectively. A low agreement between the 2+ cases by IHC and CISH was seen in most of the histologic tumor types.
Conclusions: Due to its high correlation with FISH, CISH is a useful technique to detect HER2 gene amplification in breast cancer. The assessment of Ch 17 ploidy is necessary and should be included in all low-level amplified cases. CISH and IHC showed discrepancies particularly in the 2+ category in most of the histologic tumor types of our series. For the practicing surgical pathologist, CISH for HER2 performed in conjunction with Ch 17 is a good alternative to FISH to confirm the results of IHC.

Agreement between CISH and IHC by tumor type

	IHC score		
Tumor type	0/1+	2+	3+
Lobular	3/3	0/2	-
Ductal	9/9	2/21	10/12
Pleomorphic lobular	1/1	1/1	-
Micropapillary	1/1	2/4	1/1

174 Immunohistochemical Evaluation for Myoepithelial Markers, Cytokeratin 8/18, EMA and Vimentin in the Basal-Like Subtype of Invasive Breast Carcinoma

CA Livasy, G Karaca, CM Perou. University of North Carolina, Chapel Hill, NC.

Background: DNA microarray profiling studies on invasive breast carcinomas have identified distinct subtypes of tumors that are associated with different clinical outcomes. These subtypes include luminal, normal breast-like, HER2 positive, and basal-like. The basal-like subtype is associated with poor clinical outcomes and appears common in BRCA-1 associated carcinomas. Studies attempting to identify basal-like carcinomas by immunohistochemical methods have shown that these tumors are typically ER and HER2 negative but positive for basal cytokeratins and EGFR. The purpose of this study was to evaluate for additional markers that may facilitate immunohistochemical profiling of basal-like carcinomas.

Design: Formalin-fixed, paraffin-embedded sections from 28 invasive breast carcinomas with known gene expression profiles (12 basal-like, 13 luminal, and 3 HER2 positive) were immunostained with commercially available antibodies to smooth muscle actin (SMA), p63, CD10, cytokeratin 8/18, epithelial membrane antigen (EMA) and vimentin. The degree of tumor immunoreactivity was quantitated by scoring the intensity of staining (0-3+) and the percentage of positive cells.

Results:

Table 1. Immunohistochemistry results

MA profile	SMA	p63	CD10	Cytokerat 8/18	EMA	Vimentin
Basal-like	0/12	4/12	1/12	11/12	12/12	8/12
Luminal	0/13	2/13	0/13	13/13	13/13	1/13
HER2	0/3	0/3	1/3	3/3	3/3	0/3

MA=microarray

Basal-like and luminal subtypes were essentially negative for expression of SMA and CD10. Four of 12 (25%) basal-like tumors showed weak nuclear positivity for p63; 2 of 13 (15%) of luminal tumors also showed weak nuclear positivity for p63. Cytokeratin 8/18 and EMA were expressed in nearly all tumors regardless of subtype. Vimentin was frequently expressed at a high level in basal-like carcinomas (67%) as compared to luminal (8%) and HER2 (0%). The one vimentin positive luminal tumor was only weakly positive at (2+, 10%).

Conclusions: Myoepithelial/basal markers SMA, p63 and CD10 appear to be of little value in the immunohistochemical detection of basal-like breast carcinomas. Luminal cytokeratin 8/18 and EMA are expressed in nearly all tumors regardless of subtype. Strong immunoreactivity for vimentin is seen in most basal-like carcinomas in contrast to the luminal and HER2 subtypes indicating that vimentin may be a helpful addition to the immunohistochemical panel used to identify basal-like carcinomas.

175 Diabetic Mastopathy in Patients with Non-Diabetic Autoimmune Disease

JE Love, TJ Lawton. University of Washington, Seattle, WA.

Background: Diabetic mastopathy is a histologic pattern of keloidal fibrosis, lymphocytic lobulitis, and epithelioid stromal fibroblasts that may present as a palpable breast mass. Although strongly associated with type-1 diabetes, diabetic mastopathy has been reported in non-diabetic patients, and the etiology of the lesion is unclear.

Design: We searched our case records from 2000 to 2003 and reviewed 13 cases with the histologic features of diabetic mastopathy. We reviewed the medical records of these patients, searching for histories of type-1 diabetes, type-2 diabetes, and autoimmune disorders.

Results: Of the 13 cases, 9 had a documented history of type-1 diabetes, including one case in a male patient. The 4 non-diabetic patients all had some form of autoimmune disease including Sjogren's syndrome, fibromyalgia, mixed connective tissue disease, Hashimoto's thyroiditis, and autoimmune hearing loss. None of the 4 patients had type-II diabetes.

Conclusions: In our series of 13 cases with the histologic features of diabetic mastopathy, 4 patients had no history of type-I diabetes. All 4 of these patients had an autoimmune disorder, including one patient with autoimmune sensorineural hearing loss (which, to our knowledge, has not previously been reported in the literature). This suggests that the histology of diabetic mastopathy may be better classified as an autoimmune associated finding, and not a lesion exclusive to type-I diabetes. None of the 4 patients had type-II diabetes, supporting the idea that diabetic mastopathy is not only related to elevated blood glucose. Should the histologic pattern of diabetic mastopathy be found in patients without type-1 diabetes, investigation for the presence of autoimmune disease may be warranted.

176 Columnar Cell Lesions and Flat Epithelial Atypia: Incidence and Significance in a Mammographically Screened Population

SM Lubelsky, AL Bane, V Shin, S Kulkarni, FP O'Malley. Mount Sinai Hospital, Toronto, ON, Canada; Marvelle Koffler Breast Care Centre, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.

Background: Columnar cell lesions of the breast are being increasingly recognized in biopsies performed for mammographic calcifications. Using the recently introduced WHO terminology, these lesions can be divided into columnar change/hyperplasia without atypia (CCL) and flat epithelial atypia (FEA). Using these diagnostic terms, the objective of our study was to determine the incidence and significance of these columnar lesions in a mammographically screened population.

Design: Core needle biopsies performed for mammographically detected calcifications from January 2002 to April 2004 were retrieved from the files of the Department of Pathology, Mount Sinai Hospital. Slides were reviewed detailing all pathologic abnormalities associated with calcifications and columnar lesions were categorized using the WHO terminology as described above. Follow-up excisional biopsies were also reviewed.

Results: 228 core biopsies were performed for mammographic calcifications during the study period. 4 biopsies were excluded, 3 because the biopsy was non-diagnostic and 1 because the patient had a prior history of ipsilateral breast irradiation.The 3 core biopsies with FEA had excisional biopsies performed. Two had DCIS, nuclear grade II/III, and the third case had persistent FEA.

Conclusions: Columnar cell lesions without atypia (CCL) are relatively common in breast core biopsies performed for mammographic calcifications, occurring as the dominant lesion in 21% of biopsies in this mammographically screened population. In contrast, FEA is an uncommon cause of calcifications in this series. Although the numbers are small, the association of FEA with DCIS in 2 of 3 cases suggests that excisional biopsy should be performed when a diagnosis of FEA is made in a core biopsy.

Spectrum of lesions presenting with mammographic calcifications

	IDC	DCIS	Atypical hyperplasia	FEA	Columnar Cell Lesions	PDWA/FCC	Other
Dominant lesion	10	46	23	3	47	83	13
%(n=225)	4.4	20	10.3	1.3	21	37	6

177 CK 5/6 and E-cadherin and Diagnostic Agreement in Proliferative Breast Lesions

G MacGrogan, L Arnould, S Mathoulin, A Vincent Salomon, F Bibeau, JP Ghnassia, V Picot, the GEFPICS group. Institut Bergonie; Centre Leclerc; Institut Curie; Centre Val d'Aurel; Centre Strauss; des Facteurs Pronostiques Immunohistochimiques du Cancer du Sein, France.

Background: Overall agreement in the diagnosis of non-invasive breast proliferations is decreased by the recognition of atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS). These lesions display specific CK5/6 and E-cadherin patterns of expression compared to other breast proliferations. The aim of this study was to evaluate the impact of CK5/6 and E-cadherin immunohistochemistry on the interobserver reproducibility of such lesions.

Design: 19 pathologists classified 106 cases of breast lesions in one of the following diagnostic categories : **1.**Usual Ductal Hyperplasia (UDH), **2.**Flat Epithelial Atypia (FEA), **3.**ADH, **4.**ALH, **5.**ADH+ALH, **6.**LCIS, **7.**Ductal Carcinoma In Situ (DCIS) non high grade, **8.**DCIS non high grade+LCIS, **9.**DCIS high grade, **10.**No lesion. Pathologists analyzed one HE slide of each case on a first round and one HE slide along with corresponding CK5/6 and E-cadherin immunostains on a second round. Overall and lesion -specific Kappa statistics of first and second rounds were performed.

Results: There was a slight improvement in **overall kappa values** between the first and second rounds [0.47 (SE 0.003) and 0.51(SE 0.003), respectively]. This tendency was also observed for **specific Kappa values** of lesions with characteristic CK5/6 and E-cadherin immunoprofiles between the first and second rounds ; i.e. **UDH** (0.49 and 0.61), **ADH** (0.34 and 0.39), **ALH** (0.39 and 0.43), **LCIS** (0.51 and 0.58) and **DCIS non high grade** (0.50 and 0.55). No improvement was observed for **FEA**(0.44 and 0.44) nor **DCIS high grade**(0.74 and 0.68). Interobserver disagreement was highest for cases with mixed lesions, with second round Kappa values of 0.20 and 0.37 for **ADH+ALH** and for **DCIS non high grade + LCIS**, respectively. However, Kappa values were higher and also improved between first and second rounds when lesions were grouped in diagnostic sets **A** : **no atypia, no DCIS** (0.46 and 0.59), **B** : **DCIS** (0.74 and 0.79) and **C** : **Lobular Neoplasia**(0.67 and 0.76).

Conclusions: The use of CK5/6 and E-cadherin slightly improves diagnostic agreement for specific non-invasive breast proliferations. However, overall diagnostic agreement for these lesions remains moderate. Further analysis will take into account the weight of discrepancies.

178 Expression of Focal Adhesion Protein Paxillin Is a Good Prognostic Indicator in Breast Carcinoma

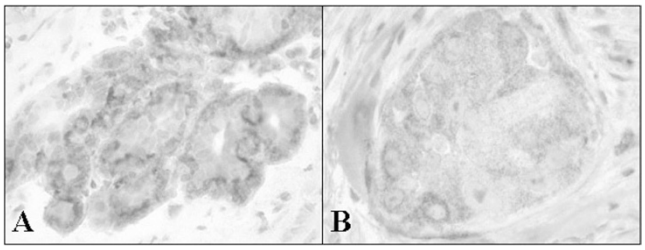
R Madan, R Cocker, K Oktay, M Oktay. Montefiore Med Ctr, Bronx, NY.

Background: Paxillin is a ubiquitously expressed focal adhesion-associated adaptor protein that plays a key role in cell spreading and motility. It is activated by phosphorylation on tyrosine upon cell adhesion to extracellular matrix or upon transformation by various oncogens. Cell motility is reduced by paxillin overexpression in lung cancer cell lines (Salgia , 2001). Low levels of paxillin have been associated with liver metastases from human colorectal carcinomas and with aggressive breast carcinomas in animals (Ayaki, 2001; Pelagalli, 2003). Our aim was to determine if paxillin can be used as a prognostic marker in human breast carcinoma.

Design: We constructed a tissue microarray with benign breast tissue (n=23) and invasive ductal carcinoma of the breast (n=72) in duplicates and analyzed expression and activation/tyrosine phosphorylation [pY^{118}] of paxillin by immunohistochemistry. Samples with moderate to strong staining were considered positive. Expression and activation of paxillin was correlated with tumor grade, lymph node metastases, lymphovascular invasion, as well as estrogen, progesterone and Her2Neu receptor positivity.

Results: In benign epithelium, paxillin expression was concentrated within myoepithelial cells while the epithelial cells showed faint or no staining (Fig. 1A). The p[Y^{118}]-paxillin was noted at the myoepithelial/basement membrane transition. Forty-seven cases (65%) of carcinomas expressed paxillin; in all, expression was localized to the cytoplasm (Fig. 1B). Paxillin activation was shown in 60% of paxillin positive cases as membrane aggregates. Expression of paxillin in carcinomas inversely correlated with lymph node metastasis (p=0.02), while activation of paxillin inversely correlated with lymphovascular invasion (p=0.01). Expression of paxillin did not correlate with tumor grade. Neither expression nor activation of paxillin correlated with estrogen, progesterone, or Her2/Neu receptor positivity.

Conclusions: Expression and activation of paxillin in invasive breast carcinomas may represent a good prognostic factor indicating low probability of lymph node metastases and lymphovascular invasion, respectively. We propose that paxillin may represent a useful prognosticator in breast carcinomas.



179 Potential Roles of T-Lymphocytes and Nature Killer Cells in Human Mammary Myoepithelial Cell Layer Disruptions and Tumor Invasion

YG Man, T Vinh, C Zhao, A Walker, R Barner. Armed Forces Institute of Pathologg, Washington, DC.

Background: The physical disruption of the basement membrane and myoepithelial (ME) basal cell layer is a pre-requisite for invasion of *in situ* breast tumors. The disruption of the basement membrane is believed to result from elevated proteolytic enzymes, while the mechanism of ME cell layer disruptions remains elusive. As our previous studies with antibodies to ME cell specific molecules and leukocyte common antigen (LCA) revealed that focal ME cell layer disruptions were consistently surrounded by or adjacent to LCA positive cells (Yousefi et al, AIMM, In press; Man and Sang. Exp Cell Res, In press; Man et al. Cancer Detect Prev, In press), this study intended to assess whether these cells belong to a cytotoxic cell population associated with ME cell layer disruptions.

Design: Consecutive tissue sections from human breast tumors (n=30) with co-existing normal, hyperplastic, *in situ*, and invasive components were double immunostained for smooth muscle actin (SMA) to identify focal ME layer disruptions, and each of the following markers, CD4, CD8, CD56, microphage, perforin, and mast cell tryptase, to elucidate the potential correlation of these molecules with focal basal layer disruptions.

Results: Multiple focal ME cell layer disruptions were seen in each of the cases, and the disruptions were consistently located at or adjacent to CD8, CD56, perforin, and mast cell tryptase positive cells. ME cells surrounded by or adjacent to these positive cells often showed a substantial reduction or total loss of SMA immunostaining and distinct signs of degenerations, compared to their counterparts away from these positive cells. Tumor cells overlying focally disrupted ME cell layers consistently displayed distinct morphological alterations in cellular density and polarity, as well as the nuclear size and shape, compared to adjacent cells within the same duct, but away from the disruption. CD4 and microphage positive cells also appeared to be associated with ME cell layer disruptions, whereas the number of these cells among cases varied substantially and the association was less consistent.

Conclusions: The consistent detection of CD8, CD56, perforin, and mast cell tryptase positive cells near ME basal cell layer disruptions suggests that these cells are likely to promote ME cell layer disruptions and tumor invasion. The development of specific agents to target these cells may have clinical value in treatment and prevention of tumor invasion.

180 Clinical Significance of Chromosome 17 Polyploidy in Patients with Breast Cancer

JR McEvoy, FM Cady, JS Metcalf, DJ Wolff. Medical University of South Carolina, Charleston, SC.

Background: Currently, the gold standard for determining HER-2 amplification (> 1 gene copy per chromosome) is fluorescence in situ hybridization (FISH). However, a pathway for over-expression of the HER-2 cell surface protein, in the absence of gene amplification (FISH negative), exists in the form of chromosome 17 polyploidy, defined in this study as cells with ≥ 3 chromosome copies in at least 33% of the 60 cells analyzed.

Design: Invasive breast cancers submitted for Her-2/neu testing by FISH were analyzed for chromosome 17 polyploidy. We sought to determine the clinical significance of chromosome 17 polyploidy in invasive breast cancers by retrospectively performing HercepTest™ immunohistochemistry (IHC) on tissue previously resulted as unamplified by FISH. Additionally, when available, data on tumor grade, tumor stage, and estrogen and progesterone receptor status were collected and analyzed.

Results: From 2001-2004, 350 consecutive cases of invasive breast cancer were analyzed by FISH, of which 248 cases had tissue available for pathologic assessment. 40/248 (16.1%) demonstrated HER-2/neu amplification using FISH, and 38 (15.3%) cases, that were unamplified by FISH, demonstrated polyploidy of chromosome 17. 35 cases with tissue available for pathologic assessement were subjected to HercepTest™ IHC, of which 14 (40%) demonstrated positive (2-3+) membranous staining. The remaining 169 cases were unamplified and non-polyploid. When available, tumor grade, tumor stage, and hormone receptor status were recorded (see table).

Conclusions: In our study, chromosome 17 polyploidy was identified in 15.3% of patients, a percentage comparable to cases demonstrating amplification. While similar in tumor grade and hormone receptor status, these cases demonstrated more advanced stage than unamplified, non-polyploid tumors. Additionally, 40% of these invasive cancers demonstrated HER-2/neu over-expression by IHC staining. We recommend HercepTest™ IHC staining on cases deemed unamplified, yet polyploid, by FISH to identify a subset of patients who may be candidates for Herceptin® therapy.

Grade, Stage, and Hormone Receptor Status

FISH	Grade I-II (%)	Grade III (%)	Stage 1-2 (%)	Stage 3-4 (%)	ER+ (%)	PR+ (%)
Amplified	22 (59)	15 (41)	24 (83)	5 (17)	22 (54)	14 (34)
Unamp/ Nonpoly	103 (73)	38 (27)	89 (95)	5 (5)	113 (67)	92 (54)
Unamp/ Poly	20 (64)	11 (36)	14 (70)	6 (30)	25 (66)	18 (47)

181 Nuclear and Cytoplasmic Expression of ß-Catenin in Spindle Cell Metaplastic Tumors of the Breast

BK McLaren, H Gobbi, P Wirth, RA Jensen, M Sanders, JF Simpson, DL Page. Vanderbilt University Medical Center, Nashville, TN; Federal University of Minas Gerias, Belo Horizonte, Brazil.

Background: Spindle cell metaplastic tumors of the breast include low grade variants with a dominant fibromatosis-like phenotype and more aggressive variants with carcinomatous or sarcomatous phenotypes. The nuclear expression of β-Catenin and associated activating or somatic mutations of the *APC/β-Catenin* pathway has been described in fibromatoses of the breast. We examined the immunohistochemical expression of β-Catenin in metaplastic lesions of the breast and fibromatosis.

Design: Metaplastic lesions and fibromatoses seen in consultation with available paraffin-embedded material were selected for immunohistochemical study. Twenty-five cases were selected: 3 fibromatosis, 9 metaplastic tumors with dominant fibromatosis-like phenotype, 8 low grade metaplastic carcinomas, and 5 intermediate or high grade metaplastic carcinomas. β-Catenin antibody (Transduction Labs, San Diego, CA) was applied at a dilution of 1:400 for one hour at room temperature following citrate antigen retrieval. Immunostaining was scored on a scale from 0 to 4+ based on percentage of cells staining, with 0 = 0-9%, 1+ = 10-24%, 2+ = 25-49%, 3+ = 50-74%, and 4+ = 75-100%. Scores of 1-4+ were considered as positive, and patterns of nuclear and cytoplasmic or membranous staining were characterized in each tumor.

Results: Sixteen of the 25 cases (64%) showed nuclear staining for β-Catenin. In the metaplastic tumors and carcinomas, nuclear staining was often confined to areas with fibromatosis-like or sarcomatous phenotype. Membranous and/or cytoplasmic staining with β-Catenin was observed in 24 of 25 cases (96%). Membranous staining was seen in epithelioid tumor cells, while cytoplasmic staining was most intense in spindled cells. The single case without cytoplasmic staining was a fibromatosis.

Staining Patterns of Metaplastic Tumors and Fibromatosis with β-Catenin

Diagnosis	Number of Cases	β-Catenin (Nuclear)	β-Catenin (Membranous or Cytoplasmic)	Fibromatosis Component
Fibromatosis	3	3	2	3
Metaplastic Tumor	9	6	9	9
Low Grade Metaplastic Carcinoma	8	3	8	4
Intermediate or High Grade Metaplastic Carcinoma	5	4	5	1

Conclusions: β-Catenin nuclear staining may be seen not only in fibromatosis of the breast, but may also be seen in the entire spectrum of metaplastic lesions. While patterns of immunostaining may provide clues to the correct diagnosis, the histologic appearance and panel of cytokeratin immunostains are necessary for accurate classification.

182 Tumor Type and Nottingham Grade of Invasive Carcinoma Following Atypical Lobular Hyperplasia on Initial Breast Biopsy

BK McLaren, PA Schuyler, M Sanders, RA Jensen, JF Simpson, WD Dupont, DL Page. Vanderbilt University Medical Center, Nashville, TN.

Background: Atypical lobular hyperplasia (ALH) is associated with a 10-20% risk of subsequent invasive carcinoma, primarily in the ipsilateral breast. According to previous studies with the Nashville Breast Cohort, these subsequent invasive tumors favor lobular features. Comparisons, including Nottingham grading and subtyping of special tumor types have not been done previously.

Design: A longitudinal follow-up study was performed on 252 women who underwent 261 benign surgical biopsies between 1950 and 1985 with a diagnosis of atypical lobular hyperplasia. Subsequent invasive breast cancers were graded and subtyped based on histologic features. Tumors of special type were compared with tumors of no special type in terms of laterality compared to side of initial ALH and death from breast cancer.

Results: Forty-eight (19%) women developed invasive breast cancer at an average 14.8 years since diagnosis of ALH. The ratio of ipsilateral/contralateral cancers compared to side of ALH was 33/11, with 1 bilateral cancer and 3 with unknown laterality. Twenty-one (44%) of the tumors were categorized as tumors of special subtypes with good prognosis. Of the tumors developing in the ipsilateral breast without concomitant ADH (30 total), 14 of these (47%) fell into these advantageous categories. At last known follow-up since cancer diagnosis (average, 12 yrs), death from breast cancer had occurred for 3 (14%) of 21 women with tumors of special type compared with 8 (31%) of 26 women with tumors of no special type (21) or an unknown type (5).

Death from Breast Cancer By Tumor Type

	Death from Breast Cancer	
Histologic Subtype	Yes	No
No Invasive Carcinoma	0	204
NST/Unknown	8	18
Special Type	3	19

Conclusions: ALH is a non-obligate precursor lesion associated with a moderate risk of breast cancer. Nearly half of the subsequent cancers show classic or variant patterns of special types with a good prognosis. The treatment of women with ALH should be influenced by their modest elevation in breast cancer risk and the good

prognosis and low mortality of many of these cancers. Less aggressive and targeted prophylactic procedures for patients with extensive forms of this premalignant condition should be considered; watchful waiting is appropriate for women with less extensive disease. With these results, clinicians may offer reassurances and reduce anxiety when counseling women with ALH.

183 Overexpression of Minichromosome Maintenance Protein 2 Is Associated with Tumor Aggressiveness and Outcome in Breast Cancer

R Mehra, S Varambally, LM Poisson, DR Rhodes, D Ghosh, AM Chinnaiyan, CG Kleer. University of Michigan, Ann Arbor, MI.

Background: Minichromosome maintenance proteins (MCM2-7) are a set of proteins essential for replication initiation and elongation in eukaryotic cells. MCM2 expression is upregulated in proliferating cells and is a marker of cell cycle entry, associated with tumor development. We sought to study the expression and potential clinical significance of MCM2 in breast cancer progression.

Design: Immunoblot analyses were carried out using normal and breast cancer whole tissue lysates and an MCM2 monoclonal antibody. Using Oncomine, a cancer microarray database (www.oncomine.org) we analyzed MCM2 mRNA levels in breast cancer. Two tissue microarrays (TMAs) were constructed using 604 tissue cores from 168 patients, comprising normal breast and the whole spectrum of breast diseases including ductal carcinoma in situ, invasive carcinomas and distant metastasis. The TMAs were immunostained with a monoclonal antibody against MCM2. Protein expression was scored taking into account intensity of staining as negative (score 1), weak (score 2), moderate (score 3) or strong (score 4); and percentage of cells expressing MCM2 using a previously validated system.

Results: By Western Blot MCM2 protein was increased in invasive carcinomas compared to normal breast. By analyzing van'tVeer's cDNA dataset using Oncomine, we found that MCM2 mRNA was elevated in grade 3 invasive carcinomas compared to grade 1 tumors (grade 3 mean=0.482, grade 1 mean= -0.469, student's t-test: t stat = -3.811, p =0.002). By immunohistochemistry, MCM2 protein expression increased with breast cancer progression. Normal breast epithelium had negative to weak staining, staining intensity increased in DCIS, and it was strongest in invasive carcinomas. High MCM2 expression was associated with features of aggressive disease including high histological grade (Fisher's exact test, p = 0.0001), ER negative (Fisher's exact test, p<0.0001) and PR negative status (Fisher's exact test, p<0.0001). High MCM2 expression was associated with younger age at diagnosis (Kruskal-Wallis test, p=0.01). Women with high MCM2 scores had shorter disease free survival rates than women with low MCM2 scores (Log rank, p =0.0007).

Conclusions: MCM2, a novel proliferation marker, increases with breast cancer progression. In patients with invasive carcinomas of the breast, higher MCM2 mRNA and protein expression is associated with aggressive breast cancer and a worse disease free survival after 10 years of follow-up.

184 GATA3 Expression in Breast Cancer: A Strong Independent Predictor of Survival

R Mehra, R Shen, S Varambally, AM Chinnaiyan, CG Kleer. University of Michigan, Ann Arbor, MI.

Background: Despite advances in detection and treatment of breast cancer, once patients develop distant metastasis, they succumb to the disease. As current prognosticators do not reliably predict which tumors will metastasize, there is a need for new markers. We previously found that EZH2 can drive malignant transformation of mammary epithelial cells and is associated with aggressive breast carcinomas. While investigating EZH2 target genes we identified GATA3, and set out to explore its role as a breast cancer biomarker.

Design: cDNA microarrays were performed on human mammary epithelial cells (HME)overexpressing EZH2 and controls. Using *Oncomine*, a cancer microarray database, we performed a meta-analysis of 4 published breast cancer expression array studies (Sorlie, van't Veer, Sotiriou, and Huang). Two tissue microarrays (TMAs) were constructed using 604 cores from 168 patients with invasive breast carcinomas. Each tumor was sampled at least in triplicate using 0.6 mm cores. The TMAs were immunostained with a monoclonal antibody against GATA3, and staining intensity was scored as negative (score=1), weak (score=2), moderate (score=3) or strong (score=4) using an internet-based tool (*Profiler*).

Results: GATA3 mRNA was decreased in EZH2 overexpressing HME cells. Metaanalysis of published breast cDNA datasets revealed that GATA3 was among 23 genes significantly associated with outcome. GATA3 mRNA was decreased in high grade tumors (grade 3 mean= -2.222, grade 1 mean=0.278, p=2.8E-6), and in ER negative tumors (ER negative mean= -0.204, ER positive =1.852, p =9.9E-6). By IHC, lower GATA3 levels were associated with larger size (p=0.03) and positive nodes (p=0.002), the strongest predictors of survival. Decreased GATA3 was associated with negative ER (p<0.001), PR (p<0.001), and with HER-2/neu overexpression (p=0.03). At the univariate level, tumors with decreased GATA3 had a worse disease free (p=0.005) and overall survival (p=0.007). GATA3 stood out as an independent predictor of survival at the multivariate level. Tumors with decreased GATA3 levels had 11.6 times higher risk of metastasis than tumors with high GATA3 (p=0.02).

Conclusions: Decreased GATA3 mRNA and protein expression is associated with larger tumors, lymph node metastasis, negative hormonal receptor status, and HER-2/neu over expression, all features of aggressive breast cancer. GATA3 is an independent predictor of survival, and if validated, its detection by IHC may be a useful test to aid clinicians in tailoring treatments for breast cancer patients.

185 Cellular Polarity in Ductal Carcinoma In Situ

SP Mehrotra, A Morimiya, B Agarwal, R Saxena, S Badve. Indiana University, Indianapolis, IN.

Background: Ductal carcinoma in situ (DCIS) has been traditionally classified into sub-types based on its architecture; however, recent classifications are based on nuclear grade (NG) alone or nuclear grade and cellular polarity (European Pathologist Working Group classification). The expression of tight junction proteins such as Zonula Occludens-1 (ZO-1) and Occludin are good markers to identify polarity in epithelial cells. ZO-1 is a membrane protein of tight junction while occludin is a trans-membrane tight junction protein. These proteins, along with adherens proteins, desmosomes and gap junctions are expressed on the apical-lateral aspects of the cell membranes. In this study we analyze the expression of ZO-1 and occludin to assess the relationship between NG and polarity in DCIS.

Design: Archival tissue specimens from 35 patients diagnosed as DCIS were analyzed for expression of ZO-1 (Zymed) and occludin (Zymed) using immunohistochemistry. Expression in adjacent normal breast epithelium served as internal positive control.

Results: The distribution of the 35 cases is as follows: three NG1; fifteen NG2, seventeen NG3; sixteen pure cribriform, ten solid, one micropapillary and 8 with mixed solid and cribriform DCIS.Comedo necrosis is observed with 8 cribriform, 5 solid and 4 mixed solid and cribriform types. The normal strong apical expression that is seen in each normal epithelial cell is lost in DCIS. Focal expression is observed in one micropapillary and 17 out of 25(68%) cribriform DCIS cases. Cells adjacent to the lumina are more likely to show persistence of tight junction proteins, though lower intensity than normal. Distribution in relation to the nuclear grade,two out of three nuclear grade1, eight of the 15 NG2 and eight of the 17 NG3 DCIS expressed ZO-1 and occludin. The vascular endothelium also showed focal polar fashion ZO-1 expression.

Conclusions: The overall expression of ZO-1 and occludin is decreased in DCIS. Persistance of tight junctions is observed in the luminal surface of the cells of cribriform DCIS. There was no correlation between nuclear grade and expression of ZO-1 and/or occludin, supporting the fact that architectural type and nuclear grade do not always go hand in hand. This is inkeeping with the Eurpean Pathologist Working Group classification which uses cellular polarity as an additional criterion to classify DCIS. Whether the persistence of cellular polarity changes the natural history of DCIS needs further analysis.

186 Microsomal Prostaglandin E_2 Synthase-1 in Breast Cancer Progression

S Mehrotra, A Morimiya, B Agarwal, R Konger, S Badve. Indiana University, Indianapolis, IN.

Background: Biosynthesis of prostaglandins is mediated by cyclooygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2). Cox-2 is expressed in 40-72 % of invasive breast cancer and is correlated with ER-negative and Her-2/neu positive status and a poor prognosis. The most, if not all, action of Cox-2 is mediated by prostaglandin E2 (PGE2) which is produced by terminal synthases, inducible microsomal prostaglandin E2 synthase-1 (mPGES-1) being one of them. Microsomal PGES-1 displays functional coupling with Cox-2 in marked preference to Cox-1. Induced expression of mPGES-1 is associated with various patho-physiological events in which Cox-2 derived PGE2 is implicated. Overxpression of mPGES-1 is seen in colorectal, non-small cell lung and endometrial cancers. The current study was designed to analyze the contribution of mPGES-1 to breast cancer progression.

Design: Archival tissue specimens from 103 cases of breast carcinoma - 32 invasive ductal carcinoma (IDC), 46 IDC and Ductal carcinoma in situ (DCIS), 5 invasive lobular carcinoma (ILC), 7 ILC and Lobular carcinoma in situ (LCIS),13 DCIS cases, and 7 benign breast cases were analyzed for the expression of mPGES-1(1:200) by immunohistochemistry and correlated the expression in various components.

Results: The expression of mPGES-1 was scored based on the intensity and percentage of area of each component stained, in every case. The scores were divided as low (1,2) and high(3,4).The distribution of the mPGES-1 is as follows:

mPGES-1 expression

Type	Normal		DCIS		Invasive	
	low	high	low	high	low	high
IDC	18	7	-	-	19	13
IDC+DCIS	27	12	16	30	19	27
ILC	3	2	-	-	1	4
ILC+LCIS	4	3	4	3	3	4
DCIS	7	2	3	10	-	-
normal	6	1	-	-	-	-

In IDC, 72% of normal lobules, 59% of invasive component, in IDC with DCIS, 69% of normal breast, 41% of IDC, 35% of DCIS, in ILC, 60% of normal, 20% of invasive, in ILC with LCIS, 42% normal, 43% of ILC, in DCIS 78% of normal, 77% of DCIS and in the benign breast cases 86% had a low mPGES-1 score.

Conclusions: The low intensity expression of mPGES-1 was observed in normal lobules of some patients with and without cancer.This expression was markedly increased in *in situ* and invasive cancers, ductal and lobular, ER positive and ER negative types. Over-expressed mPGES-1 is a common early event in breast cancer. Inhibition of mPGES -1 could be a good strategy for breast cancer chemoprevention, particularly since, unlike Cox-2, it is over-expressed in both ER-positive and ER-negative breast cancers. Some of the nonsteroidal anti-inflammatory drugs such as acetoaminophen have activity against mPGES-1 and have been shown to be chemopreventive.

187 Cyclin D1 Overexpression Is Associated with Estrogen Receptor Expression in Caucasian but Not African-American Cases of Breast Cancer

L Memeo, AK Joe, H Li, A Troxel, H Hibshoosh. Columbia University Medical Center, New York, NY; Columbia University, New York, NY.

Background: African-American (AA) women with breast cancer consistently show a shortened survival when compared with Caucasian women with breast cancer. It is not clear whether this is due to socioeconomic factors or to actual differences in tumor biology. Previous studies have demonstrated that cyclin D1 expression is strongly associated with positive estrogen receptor (ER) status in breast cancer, but these series either included primarily Caucasian patients or did not specify race or ethnicity data.

Design: In the present study we analyzed 200 cases of breast cancer from AA and Caucasian patients who were matched on age, stage, ER status, and year of diagnosis. Using immunohistochemistry, we examined the expression levels of cyclin D1, p53, p27Kip1 (p27), and p21Cip1 (p21) in these specimens. We analyzed possible racial differences in the expression of these four proteins and correlated their expression with ER status.

Results: Cyclin D1, p53, p27, and p21 expression rates were similar in matched cases of AA and Caucasian breast cancer (all p values > .05), and there were no significant differences in Her-2/neu expression, S-phase fraction, or proliferation index (MIB-1). However, we found that cyclin D1 overexpression was significantly associated with ER status in only the Caucasian (p =.0005), and not the African-American cases (p = .07).

Conclusions: This finding suggests a novel biological difference, which may be an important factor in determining the more aggressive phenotype of AA breast cancer. This difference may ultimately guide the design of specific strategies for the prevention and/or therapy of breast cancer in AA women.

188 Assessment of Variability in Diagnosing "Atypia" in Columnar Cell Lesions (CCL) of the Breast

SK Mohsin, S Badve, S Bose, CE Kleer, SE Pinder, F O'Malley. Baylor College of Medicine, Houston, TX; Indiana University, Indianapolis, IN; University of California, Los Angeles, CA; University of Michigan, Ann Arbor, MI; Addenbrooke's NHS Trust, Cambridge, United Kingdom; Mt. Sinai Hospital, Toronto, ON.

Background: CCL of the breast encompass a spectrum of proliferative lesions ranging from simple columnar change to columnar hyperplasia with or without architectural and cytological atypia. It has been suggested that a subset of CCL with "atypia" may represent an early premalignant lesion, with risks similar to atypical ductal hyperplasia (ADH). Presence of CCLs with atypia in a core needle biopsy (CNB) may have significant impact on patient care. However, the diagnostic criteria to classify these lesions as atypical are not well established. This study looked at interobserver agreement in classification and characterization of atypia in CCL.

Design: 30 cases of CCL from mammographically directed CNBs were circulated between 6 academic breast pathologists. Each participant was asked to use the criteria they employ in their routine practice and 1) classify the lesion as columnar cell change vs hyperplasia; report presence or absence of 2) architectural atypia (similar to ADH); 3) cytological atypia; and 4) if he/she would recommend excision of the mammographic abnormality based on the type of CCL in CNB. Interobserver agreement was calculated using weighted multi-rater kappa statistics with 95% confidence interval (CI).

Results:

Agreement	CCL change vs hyperplasia	Architectural atypia	Cytological atypia	Recommend excision
6/6 participants	53%	53%	40%	37%
5/6 participants	77%	67%	43%	43%
4/6 participants	97%	73%	57%	60%
Kappa (95% CI)	0.61 (0.56, 0.66)	0.53 (0.48, 0.57)	0.34 (0.28, 0.38	0.19 (0.16, 0.22)

The kappa value improved to 0.44 for cytological atypia and from 0.37 for recommending excision, if results of 5 participants were used.

Conclusions: There was moderate agreement between pathologists to classify the lesions as CCL change vs hyperplasia and recognizing architectural atypia but substantial variability for diagnosing cytological atypia in CCLs of the breast. Even when atypia was diagnosed there was a significant variability with regards to recommending excision. This degree of subjectivity and variability for recommending excision can have marked impact on patient's management, and better criteria and understanding of the significance of atypia in CCLs of the breast diagnosed in CNB is needed.

189 Are Synchronous Atypical Ductal Hyperplasia and Atypical Lobular Hyperplasia (Which Are Common) Genetically Related?

SK Mohsin, F Arbab, DC Allred. Baylor College of Medicine, Houston, TX.

Background: Atypical Ductal Hyperplasia (ADH) and atypical lobular hyperplasia (ALH) are important because they are risk factors (5-6 fold increase) and probably precursors of invasive breast cancer. We identified atypical hyperplasias in 4.5% of a recent consecutive series of 2000 core needle biopsies. Thirteen percent of the atypical cases were combined ADH and ALH. Surprised by the high incidence of combined atypias, we looked at another recent consecutive series of 2000 excisional biopsies and found 19% combined atypias among 3.75% total atypias. Although histologically dissimilar, the confirmed observation that ADH and ALH commonly coexist suggests that this subset of synchronous atypias may have an evolutionary relationship.

Design: To test this hypothesis, we evaluated the expression of two proteins (E-cadherin and beta-catenin by IHC), which are thought to have distinct expression patterns in ADH and ALH, in 64 total cases; 26 showing combined ADH and ALH, 13 pure ADH and 25 pure ALH. We assessed copy number changes by CGH after laser microdissection in 18 of 26 cases with combined ADH and ALH, in which sufficient tissue was available from both the lesions.

Results: All (100%) of the ADH, whether pure or combined with ALH, showed strong diffuse expression of E-cadherin and beta-catenin. In contrast, nearly all (85-90%) of the ALH, whether pure or combined with ADH, did not express these two proteins. Overall, allelic imbalances were common and diverse between the ADH (14 distinct gains and 10 distinct losses) and ALH (8 gains and 10 losses including 85% at 16q, which harbors the E-cadherin gene). A relatively large number of unique imbalances (15 losses and 14 gains) were found in 17 evaluable lesions (average 3.6/lesion). Six cases had informative results from adjacent ADH and ALH. Among these cases, four shared from one to five separate imbalances (representing 16% to 50% of total imbalances in each case). The phenotypes of the remaining two were dissimilar.

Conclusions: The incidence of ADH and ALH co-existing in the same breast is surprisingly high (~15%). In preliminary studies, the incidence of allelic imbalances in both types of lesions was also relatively high, and two thirds of ADH and ALH in the same breast shared imbalances, consistent with hypothesis that they may have an evolutionary relationship. An assessment (ongoing) of additional cases at higher genotypic resolution is required to confirm and better characterize this potentially important relationship.

190 Benign Spindle Cell Stromal Tumor of the Breast (BSST): A Proposed Schemata for Simplifying the Classification of Benign Myofibroblastic Tumors

PM Monteiro, BK McLaren, S Olson, RA Jensen, JF Simpson, C Leal, DL Page. Portuguese Oncology Institute, Porto, Portugal; Vanderbilt University School of Medicine, MCN, Nashville, TN.

Background: Myofibroblastomas and Myoid Hamartomas have in common a benign proliferation of spindled myofibroblastic cells in intersecting fascicles with interspersed bands of collagen. While myofibroblastomas tend to have more plump, "epithelioid" appearing cells with less glandular and adipose changes then myoid hamartomas, both tumors have variable immunoreactivity with smooth muscle markers and CD 34 and are usually immunoreactive with hormonal receptors. The two entities have broad areas of overlapping histology, and as they appear to arise from the same multipotential stromal cell, and have a similar presentation, treatment and clinical course, they should, perhaps be considered together (with other myofibroblastic proliferations) under a somewhat broader classification.

Design: Sixty-nine cases seen in consultation at VUMC (40 myofibroblastomas, 16 myoid hamartomas, and 13 additional cases given other myoid/myofibroblastic/mixed diagnosis) and 7 myoid hamartomas from the Portuguese Cancer Institute-Porto archive were characterized clinically, radiographically, and histologically. Immunostains and follow-up information were obtained when possible. An extended panel of immunostains was carefully characterized in 10 of these lesions.

Results: Seventy-three (96%) patients were women. The patients ranged in age from 27 to 87 years, with a mean age of 61 years old. The mean size of tumors was 1.87 cm (range: 0.5-6.5 cm). The majority of the tumors had pushing borders, without mitosis or atypia. Fifty-two percent and 75 % of the cases had glandular structures and fat tissue within the tumor, respectively. All (10 cases) tumors were vimentin and desmin positive; 9 were hormone receptor-positive, 4 were SMA and CD 34 negative. All were S-100 protein negative. Follow-up information was scarce, with no recurrences or metastasis.

Conclusions: Although there is a variety of histology encountered with myofibroblastic lesions, the similarity in presentation, clinical outcome, and histogenesis leads us to conclude that a grouping of such lesions under the heading benign spindle stromal tumor (BSST) may be less confusing for clinicians and convey the important message of benignancy.

191 Aberrant Promoter Methylation Profiling of Breast Cancer Using Quantitative Methylation-Specific PCR

PM Monteiro, C Jerónimo, R Henrique, AL Carvalho, MO Hoque, I Pais, C Leal, C Lopes, MR Teixeira, D Sidransky. Portuguese Oncology Institute, Porto, Portugal; Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Breast cancer is a leading healthcare concern in North America and Europe. Although the recent decline in morbidity and mortality rates associated with this disease are encouraging, further advances in early detection are needed. Indeed, a better understanding of the molecular alterations underlying this neoplasia is likely to contribute to improve diagnosis, clinical management, and outcome prediction. Aberrant promoter methylation has been described for several genes in various malignancies and the spectrum of genes involved suggests that specific tumors may have their own distinct methylation profile. In breast cancer, promoter hypermethylation has been reported for several genes, covering most aspects of cellular function, although many of them in a purely qualitative fashion.

Design: To establish a methylation profile of breast cancer, 23 gene promoters were surveyed by quantitative methylation-specific PCR (QMSP) from 66 breast carcinomas (BCa), 31 fibroadenomas (FB) and 12 normal breast tissue samples (NT). Relationships between methylation levels and clinicopathological parameters were further assessed.

Results: *CCND2*, *RASSF1A*, *CALCA*, *APC, HIN-1*, *RARβ2*, *TIG1*, and *GSTP1* displayed significantly higher methylation levels in BCa compared to FB and/or NT. Moreover, methylation levels of some genes were found to correlate with clinicopathological parameters. *CALCA, CDH1* and *BRCA1* methylation levels correlated positively with tumor grade (r = 0.26, $p = 0.03$; r = 0.42, $p = 0.0004$, and r = 0.32, $p = 0.008$, respectively). For *CCND2* and *HIN-1* the highest methylation levels were found in well differentiated tumors (r = -0.26, $P = 0.036$; r = -0.28, $P = 0.025$). Methylation levels of *APC*, *GSTP1*,

and *MT1G* correlated with tumor size (r = 0.25, *P* = 0.04; r = 0.31, *P* = 0.01; and r = 0.28, *P* = 0.02, respectively). *MT1G* methylation levels reached a statistically significant correlation with clinical stage (r = 0.31, *P* = 0.01).

Conclusions: Our data demonstrate the existence of a progressive increase of promoter methylation levels of several cancer-related genes in breast carcinogenesis and provide additional epigenetic-based markers to augment molecular detection of BCa. Furthermore, methylation levels might perfect current pathological parameters used to predict tumor aggressiveness.

192 Microsomal Prostaglandin E_2 Synthase-1 in Breast Cancer: A Possible Target for Chemoprevention

A Morimiya, S Mehrotra, B Agarwal, R Konger, S Badve. Indian Univ-Purdue Univ, Indianapolis, IN.

Background: Cyclooygenase-1 (Cox-1) and Cyclooxygenase-2 (Cox-2) play an important role in the biosynthesis of prostaglandins and related eicosanoids. Increased expression of Cox-2 is found in many human carcinomas. Cox-2 expression is seen in 40-72 % of invasive breast cancer and correlates with ER-negative and Her-2/neu positive status and therefore has a poor prognosis. Most, if not all, action of Cox-2 is mediated by prostaglandin E2 (PGE2) and microsomal prostaglandin E2 synthase-1 (mPGES-1) is involved in this process. mPGES-1 is inducible and displays functional coupling with Cox-2 in marked preference to Cox-1. Induced expression of mPGES-1 is associated with various patho-physiological events in which Cox-2 derived PGE2 is implicated and is over-expressed in colorectal, non-small cell lung and endometrial cancers. The current study was designed to analyze the contribution of mPGES-1 to carcinogenesis in invasive breast cancer.

Design: Archival tissue specimens from 89 breast carcinomas (79 ductal and 11 lobular carcinomas) were examined for the expression of Her-2/neu (Hercept Test), Cox-2 monoclonal (Cayman, 1:50) and mPGES-1 (Cayman, 1:200) by immunohistochemistry. The expression of mPGES-1 was correlated with other parameters: age at diagnosis, tumor size, lymph node status, estrogen, progesterone, Her-2 receptor and Cox-2 status.

Results: The mPGES-1 expression was seen in tumour cells in 79% cases (grade 1-77%, grade 2-82% and grade 3- 73% of cases). Expression of mPGES-1 did not correlate with with tumor size; ER status(p=0.12); Her-2 expression (p=0.12) and Cox-2 expression (p=0.41).

Conclusions: The major findings of the study are that mPGES-1 which is coupled to Cox-2 is expressed in breast cancer. We have demonstrated for the first time high levels of this enzyme in majority of breast cancers (lobular and ductal). Both Cox-2 and mPGES-1 are involved in synthesis of PGE-2 which plays an important role in initiation and progression of breast cancer.The expression of these two enzymes did not show a significant correlation and they did not always co-localize to the same tumor cells suggesting distinct underlying mechanisms. This data indicates that like Cox-2, mPGES-1 may be a target for chemoprevention.

193 Skp2 and p27 Cell Cycle Marker Expression in 155 Invasive, Poorly Differentiated Duct Carcinomas with Clinicopathologic Correlation

MP Murray, CLAubertine, E Hyjek, SJ Shin. Weill Medical College Cornell University, New York, NY.

Background: S-phase kinase-associated Skp2 is required for the ubiquitin-mediated degradation of cdk inhibitor p27. Recent studies have shown that human cancers that are high grade with poor prognosis demonstrate an inverse phenotypic expression for these markers [Skp2 positive (+)/ p27 negative (-)]. We set out to investigate Skp2 and p27 expression, separately and together, in a large cohort of patients with invasive, poorly differentiated duct carcinomas.

Design: 155 invasive duct carcinomas with an architectural and/or nuclear grade of 3 were procured from our surgical pathology files (1995-2002). Tissue microarray construction consisted of multiple 0.6mm cores of tumor as well as normal areas from each case. Using the Envision Plus peroxidase detection system (DakoCytomation), immunohistochemistry was performed on 4 µm paraffin tissue sections with mouse monoclonal antibody (Mab) anti-Skp2 (clone 2C8D9, Zymed Lab. Inc.) and Mab anti-Kip1/p27 (BD Transduction). Positive nuclear expression was determined as ≥10% and ≥50% of the total number of tumor cells evaluated for Skp2 and p27, respectively. Patient and pathology records were retrospectively reviewed for ER and HER-2/neu (H2N) expression, tumor size, lymph node status (LN), recurrence, and total follow-up.

Results: The median follow-up was 24 months (range 1-88 months). Skp2 and p27 was expressed in 43 (28%) and 102 (66%) of 155 tumors, respectively. Expression of p27 was directly related to ER status [odds ratio (OR)=3.7, 95% confidence interval (CI)=(1.8-7.5), p=0.0002]. Skp2 was positively associated with H2N protein over-expression [OR=2.4, CI=(1.2-5.0), p=0.013] and inversely correlated with ER expression [OR=4.0, CI=(1.9-8.4), p=0.0002]. No significant association was found for either marker with respect to tumor size, LN, or recurrence. 14 (9%) tumors expressed the Skp2+/p27- phenotype. This subgroup was more likely to be ER - [OR=34, CI=(6.6-177.2), p<0.0001] and H2N + [OR=9.6, CI=(2.0-46.3), p=0.001] than the subgroup of tumors that were Skp2- and p27+. No difference in recurrence rate was identified among all subgroups.

Conclusions: Skp2+/p27- tumors comprise a small subset of invasive, poorly differentiated duct carcinomas which as a group, is more likely to be ER - and H2N +. Skp2 and p27 expression of tumors may help to further sub-classify breast cancers into prognostic and biologically relevant subgroups.

194 BRAF and ERK2 Kinases in Invasive Breast Carcinoma: Absence of BRAF Mutation and Correlation of ERK2 Subcellular Distribution with Clinical Outcome

L Nakopoulou, E Mylona, P Rafailides, P Alexandrou, A Seata, Ch Apostolopoulos, S Markaki, A Keramopoulos. Medical School, National and Kapodistrian University of Athens, Athens, Greece; Alexandra Hospital, Athens, Greece.

Background: MAP kinase pathway represents a cascade of phosphorylation events including three pivotal kinases, namely Raf, MEK and ERK1/2 which has been implicated in the pathogenesis of cancer. The family of Raf kinases consists of ARAF, BRAF and c-RAF-1. The purpose of the present study was to investigate the possible mutations in BRAF gene and the expression of ERK2 protein in relation to the classic clinicopathological parameters, markers such as ER/PR and patients' survival.

Design: The immunohistochemical method ABC-HRP was performed in paraffin-embedded tissue specimens from 151 invasive breast carcinomas to detect the proteins ERK2, ER/PR. In frozen tissue of 52 of these specimens PCR-SSCP was applied to detect possible BRAF gene mutations.

Results: No mutation at exon 15 of BRAF gene was observed. ERK2 protein was detected in the cytoplasm and the nucleus of malignant cells in 37.7% and 19.2% of the cases, respectively. Nuclear ERK2 was inversely correlated with ER (p=0.039), whereas cytoplasmic ERK2 was more often detected in lobular carcinomas (p=0.026). Nuclear ERK2 was found to be an independent prognostic factor of a shortened overall survival of the patients (p=0.040), while cytoplasmic ERK2 had an independent favorable effect on patients' both disease-free and overall survival (p<0.001 and p=0.002 respectively).

Conclusions: The present study indicates that compartmentalization of ERK2 reflects a different effect on clinical outcome, with nuclear ERK2 to be prognostic of a shortened overall and cytoplasmic to be an independent, favorable prognosticator of both disease-free and overall survival. Furthermore, BRAF mutations do not seem to be involved in breast cancer development or to be responsible for the detected ERK2 hyperexpression.

195 Expression of p27 in Primary Versus Metastatic Breast Carcinomas: Its Prognostic Significance and Relation to Other Prognostic Markers

AL Namiq, OW Tawfik, F Fan, PA Thomas. Department of Pathology, Kansas City, KS.

Background: p27 protein, is a cyclin-dependent kinase inhibitor, plays an important role in regulating the progression of cells from G1 into S phase of the cell cycle. In breast carcinoma, low p27 expression has been correlated with poor outcome and tumor progression. In this study we evaluate p27 expression in primary breast carcinomas and their paired lymph nodes metastases. We further analyze the potential relationship between p27 expression and other prognostic markers including estrogen receptor (ER), progesterone receptor (PR), Her-2-Neu, EGFR, Ki-67, p53 and Bcl-2.

Design: Immunohistochemical studies for p27 were performed on 15 primary breast carcinomas and their paired lymph nodes metastases (10 invasive ductal carcinomas; 3 invasive lobular carcinomas; 1 mixed ductal and lobular carcinoma; 1 metaplastic carcinoma). Tumor sizes and histological grades were recorded. Patients' age ranged from 29-89 years old. The cut-off percentage for the marker p27 to be considered positive was >50%. ER, PR, Her-2-Neu, EGFR, Ki-67, p53 and Bcl-2 were also evaluated on primary and metastatic breast carcinomas for those 15 cases and their relationship to p27 expression was examined.

Results: Almost all metastatic deposits; 14/15 cases (93%) lost p27 expression. Significant difference was found in p27 expression in primary and metastatic breast cancers. 9 /15 (60%) of the primary breast cancers that were positive for p27 lost the marker when they metastasized, 5/15 (33%) cases showed no p27 expression in both primary and metastatic breast cancers. 1/15 (7%) retained p27 expression in both primary and metastatic breast lesion. However, no gain of p27 expression in metastases. ER, PR, Her-2-Neu, EGFR, Ki-67, p53, and Bcl-2 had almost an identical pattern of expression in primary and metastatic lesions. No correlation was observed between p27 expression and these prognostic markers.

Conclusions: The vast majority of metastatic breast carcinoma deposits showed loss of p27 expression. Discordance in p27 expression was noted in primary compared to metastatic breast lesions. The majority of tumors lost their p27 expression once they metastasized.

No correlation existed between p27 expression and hormone receptor status, and proliferative activity or the expression of Her-2-Neu, EGFR, p53, and Bcl-2.

The loss in p27 expression in metastatic breast carcinoma deposits is illustrative of acquisition or expression of more aggressive behavior required for metastasis and tumor heterogeneity.

196 15-LOX and COX-2 Expression in Breast Cancer: Correlation with Estrogen Receptor and Prognosis

A Nassar, A Radhakrishnan, IA Cabrero, GA Cotsonis, C Cohen. Emory University School of Medicine, Atlanta, GA; Mexican Oncology Hospital, Mexico; Emory University School of Public Health, Atlanta, GA.

Background: Lipoxygenases and cycloxygenases are mediators of arachidonic acid metabolism. The eicosanoid metabolites from these oxygenases regulate the growth and death of cancer cells. Recently, studies have reported that human breast carcinomas aberrantly express lipoxygenases and cyclooxgenase-2 (COX-2) and that decreased levels of 15-lipoxygenase (15-LOX) and raised levels of COX-2 and 12-LOX have prognostic value in patients with breast cancer. 15-LOX was significantly reduced with increasing stage, and in patients who developed metastatic disease, local recurrence, and/or died. With high COX-2, patients developed local recurrence, died of breast cancer and had reduced disease-free and disease-related survival in estrogen

receptor (ER)-negative but not ER-positive disease. COX-2 expression is also associated with increased angiogenesis, lymph node metastasis and Her2 neu overexpression.
Design: Five tissue microarrays (TMA) were constructed from 44 breast carcinomas and five normal breast tissues, represented by 1 mm cores in triplicate from each of three foci. TMA cores (nine from each carcinoma) were immunostained with 15-LOX (1/1600), COX-2 (1/200), and ER (1/50). Expression was assessed as intensity and scored as percent of cells positive. Prognostic parameters and follow-up information were obtained from the hospital records of Mexican Oncology Hospital, Mexico, where the carcinomas were diagnosed.
Results: All (100%) of the breast carcinomas showed cytoplasmic 15-LOX expression (43/43), cytoplasmic COX-2 expression (41/41), and nuclear ER expression (27/27). Because of lack of variability in 15-LOX (all positive, score 4), no statistical analysis could be performed on this cohort. COX-2 intensity and percentage of cells positive correlated significantly with histologic grade ($p = 0.0245$; $p = 0.0498$) and size of carcinoma ($p = 0.0035$; $p = 0.0288$). ER intensity and score significantly associated with histologic grade ($p = 0.0075$; $p = 0.0003$). Neither COX-2 nor ER correlated with follow-up. There was no significant correlation between COX-2 and ER.
Conclusions: 15-LOX is overexpressed in the majority of breast carcinoma. COX-2 and ER correlated significantly with high histologic grade. COX-2 also correlated significantly with tumor size. Neither COX-2 nor ER correlated with outcome (overall survival and disease-free survival).

197 How Repoducible Is the Diagnosis of Flat Epithelial Atypia of the Breast?

FP O'Malley, S Badve, S Bose, LC Collins, M Ennis, CG Kleer, SK Mohsin, SE Pinder, SJ Schnitt. Mount Sinai Hospital, Toronto, ON; Indiana University, Indianapolis, IN; Cedars Sinai Medical Center, Los Angeles, CA; Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA; University of Toronto, Toronto, ON; University Hospital, Ann Arbor, MI; Baylor College of Medicine, Houston, TX; Addenbrooke's NHS Trust, Cambridge, UK.
Background: Lesions of the breast in which epithelial cells of terminal duct lobular units are replaced by one to several layers of columnar cells with low grade cytologic atypia are being identified increasingly in biopsies performed because of mammographic microcalcifications. TheWHO Working Group on the Pathology and Genetics of Tumours of the Breast (2003) introduced the term "flat epithelial atypia" (FEA) for these lesions. However, the ability of pathologists to reproducibly diagnose FEA, and to distinguish it from columnar cell lesions without atypia, has not been previously evaluated.
Design: Eight pathologists with an interest in breast pathology participated in a study to address this issue. The study reference pathologist (SJS) provided the other 7 study pathologists with a Powerpoint tutorial that included written criteria for FEA and columnar cell lesions without atypia, and images from representative examples of each category of lesion. Following review of the tutorial, the study pathologists examined images from 30 cases of FEA and columnar cell lesions without atypia, also provided as a Powerpoint file, and were instructed to categorize each lesion as either "FEA" or "not atypical."
Results: Overall agreement among the 8 pathologists for the 30 cases was 91.8% (95% CI, 84.0-96.9%), and the multi-rater kappa value was 0.83 (95% CI, 0.67 – 0.94), which is within the "excellent agreement" range. Complete agreement among all 8 pathologists was achieved in 24 cases (80.0%), at least 7 pathologists agreed in 26 cases (86.7%), and 6 or more agreed in 28 cases (93.3%). Agreement was slightly better for determining absence of FEA (92.8% [95% CI, 84.1%-97.4%]), than for determining presence of FEA (90.4% [95% CI, 79.9%-96.7%]).
Conclusions: We found that the diagnosis of FEA and its distinction from columnar cell lesions without atypia is highly reproducible with the use of standardized criteria. Uniform application of these criteria should foster inter-observer agreement in the clinical setting and should enable more accurate comparisons of research studies evaluating the clinical significance and biological nature of these lesions.

198 Clinicopathological Relevance of Akt, Bad, Caspase-3, and Ki-67 in Breast Carcinoma Following Neoadjuvant Chemotherapy

G Peiro, E Adrover, FI Aranda, C Alenda, MF Peiro, A Paya, J Segui. Hospital General Universitari, Alacant, Spain.
Background: Neoadjuvant chemotherapy (NACT) represents the first indication for the management of high risk patients with breast carcinoma (BC). Apparently, it improves the rate of operability, breast conserving surgery, local control and outcome. However, the role of biological factors that can predict response to this modality of treatment is unknown
Design: We selected 98 core needle biopsies (CNB) and the corresponding resection specimens from BC patients in NACT. Clinical response (CR) based on tumor size was classified as complete (100%), partial incomplete (>50%), minor (<50%) and progression. Tumor cellularity was assessed from H&E sections in CNB and specimens as the percentage of tumor area with invasive cells. Surgical specimens were classified using a five-point grading system (Miller/Payne). Immunohistochemistry (IHC) for phosphoAkt (pAkt Ser473), phosphoBad (pBad Ser136), cleaved Caspase-3 and Ki-67 was performed in CNB. Staining for pAkt and pBad was considered as negative (no/faint) or positive (moderate/strong) of cytoplasm/nuclei staining. Cleaved Caspase-3 and Ki-67 were scored based on the proportion. Results were correlated with CR and pathologic response (PR), and several clinical pathological factors.
Results: Median age of the patients was 48 years (range 25-81). Median initial tumor size was 5 cm (range 1-14) and after treatment 2.3 cm (range 0-22) (p=0.017). 87% were of ductal type, 13% lobular and 60% grade 3. pAkt was negative in 43% and pBad in 57% tumors. Activated caspase-3 in ≥50% cells was seen in 17% and Ki-67 >30% in 27%. Tumor cellularity significantly decreased from a median of 30% to 5%, and 30% showed >90% reduction of the clinical size (p<0.000), which correlated with Ki-67 levels (p=0.05). pAkt was more frequently negative in tumors with Ki-67 <30% (p=0.001). Complete CR and PR was seen in 34% and 19%, respectively (p=0.002). Better CR was observed in ductal carcinomas (p=0.017), with negative pAkt (p=0.037), Ki-67 >30% (p=0.01), and as a trend with pBad (p=0.09). However, the grade of PR correlated only with histologic type (p<0.000), the presence of cytological changes (p=0.012), and as a trend with Ki-67 (p=0.11)
Conclusions: In BC following NACT, activated Akt and Ki-67 levels correlate with the CR. Tumor cellularity and size are significantly reduced, but the PR do not depend on the levels of pAkt, pBad, or active caspase-3. Among conventional pathologic factors, only the type of the tumor predicts both responses
Supported by grant (FIS 03/1411)

199 Detection of Human Papilloma Virus (HPV) Sequences in Breast Cancer Samples of Mexican Patients

MD Perez-Montiel, DF Cantu de Leon, I Mikyskova, TA Vela, JG Chanona Vilchis, R Pichardo, HR Dominguez-Malagon, O Hes. Instituto Nacional de Cancerologia, Mexico City, Mexico; University of Pilzen, Pilzen, Czech Republic; Hospital Medica Sur, Mexico City, Mexico.
Background: Breast cancer is the second most common female cancer in México, (10,000 new cases every year). Some classical risk factors are well recognized ; nevertheless up to 80% of patients with this tumor do not have any recognizable risk factor. In the world, there is some evidence of an association between breast cancer and viruses, such as human papilloma virus and mouse mammary tumor virus (MMTV). At present time there is no report of detection of HPV sequences in mexican patients with breast cancer.
Design: From January 1999 to December 2003, 65 cases of breast cancer were selected, one representative paraffin block was selected, HPV DNA was analyzed by polymerase chain reaction (PCR) and sequenced for different types of HPV. Of all selected cases 51 specimens showed DNA integrity by b-globin amplification. Descriptive analysis of clinical and pathological variables was performed and comparisons between positive and negative cases were done by chi-square test and student-*t*. Statistical significance was accepted at the 5% level.
Results: Mean age was 53.3 ± 13.2 years (range 27-82), cervicovaginal cytology was evaluated and only 1 patient (1.5%) had HPV, and no cases showed cervical dysplasia. 60 tumors (92.3%) were ductal carcinomas, tumor size ranged 1-17 cms (mean 9.4cms), Scarff-Bloom-Richardson index of 8 and 9 was found in 11 (16.9%) and 10 (15.4%) cases respectively. Estrogen receptors were positive in 15 cases (23%) and progesterone receptors were positive in 16 (24.6%). Of the 51 patients with intact DNA 36 (70.5%) were negative to HVP and 15 (29.4%) were positive, when viral typing was performed, 10 (66.6%) were positive for HPV 16, 3(20%) for HPV 18, and two cases (13.3%) were positive for HPV 16 and 18. When comparison between HPV positive and negative were done in relation to all clinical and pathological variables the only significant one was positive HPV and small tumor size (p=0.008).
Conclusions: A high prevalence of HPV in breast tumors in Mexican woman was found , similar to other reports in the literature, the most common type was HPV 16. Tumors larger than 4 cms rarely have HPV DNA, this feature is similar to large carcinomas of uterine cervix. Study of a larger series of cases need to be analyzed in order to establish the exact role of HPV virus in the pathogenesis of breast cancer.

200 Epidermal Growth Factor Receptor (EGFR) Expression in High Grade Invasive Mammary Carcinoma

ML Policarpio-Nicolas, KT Schafernak, LK Diaz, N Kidwai, EL Wiley. Feinberg School of Medicine Northwestern University, Chicago, IL.
Background: EGFR is a cell surface protein with extracellular, transmembrane and intracellular domains responsible for activating multiple downstream signaling pathways resulting in the expression of genes involved in tumor growth and progression. EGFR belongs to a family of molecules that includes HER2. Expression of HER2 has been associated with aggressive, high grade mammary cancer. Another subset of breast tumors with an aggressive clinical course are high grade, estrogen receptor negative, HER2 negative cancers (ER-/HER2-), which are frequently positive for mutated p53. EGFR expression by these ER-/HER2- breast tumors has not been well characterized. The aim of this study is to 1) to determine the rate of EGFR expression for high grade breast carcinoma; and, 2) to determine if there is a relationship between EGFR expression and breast biomarkers ER, HER2 and p53.
Design: Tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded high grade infiltrating breast carcinoma cases. Immunohistochemical stains were performed using antibodies for ER, p53, HER2, and EGFR (Dako) and detected using the iView system (Ventana). The stained sections were scored by two pathologists using a two-tiered grading scheme (negative or positive).
Results: 216 tumors were evaluable: 103 were post-chemotherapy cases, 80% grade 3, 132 stage T2 or T3, and 128 were lymph node positive tumors. Of these 216 cases, we found: 57 EGFR+, 125 ER+, 106 p53+, and 75 HER2+. EGFR was found to be expressed independent of tumor size, lymph node status, HER2 status and residual tumor burden following neoadjuvant chemotherapy. The table summarizes biomarker data for ER, p53 and HER2.

EGFR Compared to Other Markers

EGFR	ER+	ER-	p53+	p53-	HER2+	HER2-
Positive	11	44	35 (b)	19	15 (c)	41
Negative	114 (a)	47	71	88	60	99

(a) p<.001; (b) p<.01; (c) p=.02

Conclusions: These results demonstrate a significant inverse relationship of EGFR expression to ER expression and a significant positive correlation with immunoreactive p53 protein expression for high grade mammary carcinoma. We found the overall incidence of EGFR expression to be about 1/4th of tumors (26%) in this patient cohort. EGFR expression is more frequently observed in the ER-/HER2- group of breast tumors of which a subset coexpresses p53.

201 Telomere Length Changes in DCIS and Recurrent Disease

ML Policarpio-Nicolas, EL Wiley, S Kulkarni, V Papavero, SA Khan, LK Diaz. Northwestern University, Chicago, IL.

Background: Shortened telomeres have been observed in most human epithelial cancers and critical shortening is associated with chromosomal instability. Recently, Meeker et al. analyzed telomere lengths in archival breast cancer cases (Am J Pathol 2004;164:925-35) using a newly developed method of telomere length assessment (TEL-FISH) on paraffin sections. Telomeric shortening was seen in the majority of invasive breast cancer cases and elongated telomeres were observed in a small subset of cases. Telomeric shortening was also seen in DCIS. We sought to further investigate telomere length changes in DCIS by performing TEL-FISH on cases of DCIS from patients who went on to develop recurrent disease.

Design: Tissue microarrays containing 10 primary DCIS cases, matched recurrences, and 10 controls (non-recurrent DCIS cases) were used. DCIS cases were grade 1 (2 cases), grade 2 (6 cases), and grade 3 (2 cases). Invasive cancer represented the recurrent disease in 2 cases; DCIS in 8 cases. The TEL-FISH procedure was performed as previously described (Am J Pathol 2002;160:1259-68). The procedure utilizes a cy3 labelled, telomere specific, peptide nucleic acid probe. The cases were scored manually using a fluorescent microscope. Luminal epithelial cells (from benign reduction mammoplasty specimens) and myoeptihelial cells were utilized as controls to establish normal reference ranges for telomeric signal numbers.

Results: Marked telomeric signal reduction was observed in eight primary DCIS cases and one case displayed near normal telomeric signals. Interestingly, markedly increased telomeric signals were seen in one case (as previously observed in invasive breast cancer). In 2 cases, a marked increase in telomeric signals was seen in the recurrent compared to the primary DCIS. In one case, a marked reduction in telomeric signals was observed in progression to invasive disease. In 7 cases, telomeric signals of primary and recurrent disease were relatively similar. No appreciable differences in telomeric signals were observed in the control cases.

Conclusions: Patterns of telomere length variation in DCIS appear to be similar to those observed for invasive breast cancer with the majority of cases possessing shortened telomeres and occasional cases with near normal and elongated telomeres. Telomere lengths appear to remain stable during DCIS progression; however, both increases and decreases do occur in a minority of cases.

202 Clinicopathologic Analysis of Solid Papillary Carcinoma of the Breast

H Qureshi, H Nassar, D Visscher. Mayo Clinic, Rochester, MN; Wayne State University/Harper Hospital, Detroit, MI.

Background: Solid papillary carcinomas (SPC) are uncommon tumors, described by Maluf et al, that often exhibit neuroendocrine features and are frequently accompanied by colloid carcinoma. They are composed of crowded but circumscribed cellular nodules separated by bands of dense fibrosis with little or no intervening breast tissue. It is unclear whether SPC represent purely in situ lesions. The aim of the study is to describe the pathological features and outcome of 50 SPC (mean follow up 8yr).

Design: Cases were divided based on histologic evaluation into groups of SPC only (36%) and SPC with invasive components, consisting of colloid-44%, carcinoid-like-16%(where neoplastic cells grow in trabeculae and nests separated by a richly vascular stroma); alveolar/nested-9% (where small and large nests of neoplastic cells sometimes indiscernible from ductal carcinoma in situ invade the breast parenchyma and fat with no or very little desmoplasia), ductal NOS-3 %, lobular-3%, tubular-3%, or mixed-29% (mixed colloid with one of the other patterns).

Results: The mean age was 73 years. All were estrogen receptor positive and 86% were Grade 1. The tumor measured <0.5cm in 13%, 0.5 to 1.0 cm in 28%, 1.0 to 2cm in 15%, and >2cm in 44%. In 37.5%, the invasive carcinoma was multifocal. Axillary nodes were involved in 14% of the cases; all of these had an invasive component in the primary tumor (2 carcinoid-like, 1 alveolar/nested, 2 mixed). Overall, 5/45 patients (11%) died with distant metastases; one of these (20%) had positive nodes and all had invasive components (colloid-3, lobular- 1, alveolar/nested-1).

Conclusions: SPC are heterogeneous lesions that arise in older women and have an indolent behavior. Lymph node and distant metastases are uncommon and limited to cases with (conventional) invasive components.

203 Cytokeratin 5/6 Distinguishes Solid Papillary Ductal Carcinoma In-Situ from Florid Usual Ductal Hyperplasia

JT Rabban, FC Koerner, MF Lerwill. Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Background: The solid papillary variant of ductal carcinoma in situ (SP-DCIS) can be difficult to distinguish from florid usual ductal hyperplasia (UDH) involving a papilloma, as the two lesions have many similar morphologic characteristics. Recent studies have shown that 96-100% of conventional DCIS is negative for cytokeratin 5/6 (CK5/6), whereas 88-100% of UDH is positive, suggesting that this antibody may also be helpful in distinguishing SP-DCIS from UDH. Specific examination of CK5/6 staining in SP-DCIS, however, has not been reported to date.

Design: Fourteen cases of SP-DCIS and nine cases of florid UDH were selected from departmental files. Immunohistochemical staining on formalin-fixed, paraffin-embedded tissue was performed using antibody clone D5/16B4 (DAKO, 1:40) against cytokeratin 5/6 and a Ventana automated processing system. Cytoplasmic or membrane staining was considered positive.

Results: Normal myoepithelium and scattered luminal epithelial cells in unremarkable ducts and acini showed variable expression of CK5/6. The proliferative cells in all cases of UDH, including 4 involving papillomas, showed strong staining for CK5/6. The percentage of positive hyperplastic cells ranged from 50-80%, although the majority of cases (77%) demonstrated at least 80% positivity. The positive cells were evenly distributed throughout the intraluminal proliferation. In 8 cases (57%) of SP-DCIS, all the tumor cells were negative for CK5/6; in the other 6 (43%), <5% of the tumor cells were weakly positive. Residual benign ductal epithelial cells and myoepithelial cells entrapped in foci of SP-DCIS were also frequently positive for CK5/6.

Conclusions: CK5/6 is strongly expressed in at least half of the cells in florid UDH. In most cases, greater than 80% of the proliferative cells are positive. These results contrast with those found in SP-DCIS, which shows a complete absence of staining or only <5% weak positivity for CK5/6. Therefore CK5/6 can be useful for distinguishing florid UDH from SP-DCIS. A positive immunoreaction in the majority of cells supports the former diagnosis and rare positivity or a negative result supports the latter.

204 Intratumoral Heterogeneity of Immunohistochemical Marker Expression in Breast Carcinoma: A Tissue Microarray Based Study

A Radhakrishnan, A Nassar, IA Cabrero, G Cotsonis, L Lyons, C Cohen. Emory University School of Medicine, Atlanta, GA; Mexican Oncology Hospital, Col Roma, Mexico; Emory University School of Public Health, Atlanta, GA.

Background: Needle core biopsies of breast carcinomas provide diagnostic, prognostic, and predictive information prior to neo-adjuvant therapy. Possible intratumoral heterogeneity of biomarker expression questions validity of their interpretation in small biopsies. Using tissue microarray (TMA) technology, we studied intratumoral heterogeneity of seven immunomarkers.

Design: Five TMA's were constructed from 44 breast carcinomas and 5 normal breast tissues, represented by 1 mm cores in triplicate from each of three foci .TMAs were immunostained for monoclonal Estrogen Receptor (ER)(1/50), monoclonal Progesterone Receptor (PR)(1/40), polyclonal Her2neu (1/200), monoclonal E-Cadherin (E-Cad)(1/200), monoclonal Epidermal growth factor receptor (EGFR) (RTU), monoclonal P53 (1/80), and monoclonal MIB-1(1/160). Expression was quantified visually and by image cytometry as intensity, percentage of cells positive, and score.

Results: Using intraclass correlation coefficient (ICC), heterogeneity in the expression of the immunomarkers within subjects was compared to the overall variance. The higher the percent, the smaller the within variance as compared to the total; the lower the percent, the greater the heterogeneity within the class. There was intratumoral heterogeneity in the following immunomarkers:

Table 1

Immunomarkers (% positive)	Image/visual	Intensity, percentage and /or score	ICC%
ER (45)	image	intensity/score	40.93%/39.87%
PR (38.6)	image	score	39.56%
PR	visual	intensity	0.00%
Her2neu (25)	visual	percentage/score	19.89%/22.42%
P53 (31)	image	intensity/score	40.14%/39.37%
P53	visual	percentage/score	4%/4.2%
MIB-1	image	score	39.15%

E-cad (45%) and EGFR (6%) failed to show intratumoral heterogeneity. MIB-1 labeling index was low (<8%) in 44.2%, intermediate (8-12%) in 23.2%, and high (>12%) in 32.6%.

Conclusions: Intratumoral heterogeneity in ER, PR, Her2neu, P53, and MIB-1 indicates their problematic interpretation in small biopsies as indicative of the status of the entire carcinoma. A negative result does not exclude the expression of these markers in the rest of the tumor. E-cad (positive in ductal carcinomas), and EGFR lacked heterogeneity.

205 Cyclin E1 and Survivin mRNA Levels Compared in Normal Breast, Hyperplasia and DCIS from Archival Tissue

V Rausei, RC Chan, WS Nichols, S Bose. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Aberrant expression of Cyclin E1, a cell cycle regulator, and survivin, an anti-apoptosis protein have been observed in invasive breast carcinoma. Expression levels correlate with prognosis. In order to determine the role of these proteins in breast carcinogenesis, we evaluated their mRNA levels in early proliferative lesions of the breast, ductal hyperplasia and ductal carcinoma in situ (DCIS).

Design: 16 cases each of DCIS and hyperplasia and 20 normal breasts from reduction mammoplasties were retrieved from pathology archives. This tissue had been prepared by routine fixation in 10% neutral buffered formalin and paraffin-embedded. Sections were cut at 4 microns and 1000 to 1500 epithelial cells microdissected for each case using laser capture dissection (LCD). Total RNA was isolated from dissected cells. Cyclin E1, Survivin and house keeping gene (GAPDH) mRNA levels were determined by real-time RT-PCR, and relative Cyclin E1 and Survivin expression established, for each case.

Results: mRNA of both cyclin E1 and survivin was detectable in normal breast epithelium, hyperplasia and DCIS. Relative values are presented below. Although a range of expression levels were noted in each of the three categories, there was a progressive increase in expression from normal to DCIS. DCIS showed a greater than 2-fold increase in mean expression over normal and hyperplasia. Additionally, 3 cases of DCIS showed more than a 5-fold increase in cyclin E1 and/or survivin level. Interestingly, all three of these cases showed high nuclear grade and comedo necrosis.

Relative Cyclin E1 and Survivin mRNA in Breast Proliferative Lesions:

	Cyclin E		Survivin	
	% pos	Mean (Range)	% pos	Mean (Range)
Normal	50	1.1 (0-3.4)	35	0.2 (0-1.1)
Hyperplasia	50	1.4 (0-6.6)	56	0.5 (0-3.1)
DCIS	85	3.7 (0-14.6)	77	2.6 (0-15.9)

Conclusions: mRNA of both Cyclin E1 and Survivin is detectable in low levels in normal breast tissue, progressively increasing in hyperplasia through DCIS. DCIS shows a greater than 2-fold increase in levels of both cyclin E1 and survivin; these are heterogeneous levels in DCIS and could be useful as diagnostic and/or prognostic factors. Further studies are merited for elucidation of the role of these two pro-survival pathways in DCIS.

206 Are Metaplastic Breast Carcinomas Basal-Like Tumours?

JS Reis-Filho, F Milanezi, P Simpson, LG Fulford, D Steele, J Nesland, E Pereira, SR Lakhani, F Schmitt. ICR, London, United Kingdom; IPATIMUP, Porto, Portugal; The Radium Hospital, Montebello, Norway; Laboratório Salomão & Zoppi, Sao Paulo, Brazil; University of Porto, Porto, Portugal.

Background: It has been suggested by our group and others that the majority of metaplastic breast carcinomas (MBCs) show features of basal/ myoepithelial differentiation. Recently, an immunohistochemical panel comprising antibodies against oestrogen receptor (ER), HER-1(EGFR), HER-2 and cytokeratin (Ck) 5/6 was reported to successfully identify basal-like breast carcinomas, as defined by cDNA microarrays. Our aim was to analyse a series of 60 MBCs using this panel plus progesterone receptor (PgR) and two other basal markers (Ck 14 and p63), to further define how frequently MBCs show a basal-like immunophenotype.

Design: 60 cases were retrieved from the pathology archives of the authors' institutions and reviewed by 4 authors. The tumours were reclassified into 4 groups: MBC with heterologous elements, MBC with squamous metaplasia, spindle cell carcinomas and matrix producing breast carcinomas. Immunohistochemistry with antibodies for ER, PgR, HER-1, HER-2, Ck 5/6, Ck14 and p63 was performed according to standard methods and semi-quantitatively scored by 3 of the authors into three categories: negative, < 1% of positive neoplastic cells, +, 1 - 10% of positive neoplastic cells, ++, >10% of positive neoplastic cells. HER-2 was scored according to the guidelines for herceptest.

Results: Table 1 summarises the immunohistochemical profile of the cases. All but four cases showed the typical immunoprofile of basal-like tumours (ER and HER-2 negative, HER-1 and/or Ck 5/6 positive). PgR showed positivity in 21.3% of squamous cell carcinomas, whereas ER was positive in only 10%. In addition, the other two basal markers Ck 14 and p63 were positive in 84.3% and 75% of the cases, respectively.

Conclusions: Regardless of the type of metaplastic elements, the vast majority (93.3%) of MBCs show a basal-like phenotype. As these neoplasms frequently overexpress HER-1 (79.6%), patients with MBC may benefit from treatment with anti-HER-1 drugs (therapeutic antibodies or small molecule tyrosine kinase inhibitors).

Cases	MBC with heterologous metaplasia				MBC with squamous metaplasia				Matrix producing carcinoma				Spindle cell carcinoma			
	N	- (%)	+ (%)	++ (%)	N	- (%)	+ (%)	++ (%)	N	- (%)	+ (%)	++ (%)	N	- (%)	+ (%)	++ (%)
ER	6	100	0	0	20	90	10	0	13	100	0	0	15	93.3	0	6.7
HER-2	6	0	0	0	23	95.6	0	4.3	14	100	0	0	15	100	0	0
Ck 5/6	6	33.3	16.7	50	24	4.2	8.4	87.5	13	15.4	7.7	77	16	50	0	50
HER-1	5	16.7	16.7	50	21	9.5	0	90.5	9	22.2	22.2	55.6	15	35.7	7.1	57.1
PgR	6	100	0	0	23	78.3	4.3	17.4	14	100	0	0	16	93.7	0	6.2
Ck 14	6	16.7	16.7	66.7	24	16.7	4.2	79.2	13	23.1	0	76.9	16	50	0	50
p63	6	0	16.7	3.3	24	25	0	75	14	37.3	28.6	37.7	16	25	0	75

207 Identification of the Relative Levels of Human Cytokeratins Expression in Adenocarcinoma of the Breast: Comparison with 60 Different Tumor Types

M Reyes, D Baunoch, MW Moore, BW Tirtorahardjo, M Opel, A Au, G Krause, A Tassin, N Edejer, J Haut, X Ma, M Erlander. US Labs, Irvine, CA; Arcturus, Mountain View, CA.

Background: Gene expression profiling is a powerful new technology capable of measuring the relative levels of transcription for genome wide gene expression. Using gene expression profiling we have evaluated the relative levels of expression of human cytokeratin and cytokeratin-associated genes in breast tumors across 550 tumors, representing over 60 tumor types.

Design: For each case, a single 5um section was stained (H&E), and the tumor visualized. Pure tumor populations were obtained by either manual dissection, or laser capture microdissection (Arcturus, Mountain View, CA). Samples were processed using a silica spin column-based extraction method (Arcturus, Mountain View, CA). The total quantity of RNA extracted was assessed using quantitative PCR (Taqman, ABI), with primers specific for B-actin transcription. Only samples with greater than 10ng of RNA were amplified. Samples were amplified using a modified RNA polymerase 2-round amplification protocol (Arcturus, Mountain View, CA). Following amplification, the RNA product yield was quantitated by OD (260/280) spectroscopy and the amplified product visualized using denaturing electrophoresis on a 1.5% agarose gel. Resulting sample RNA was labeled with Cy5, through conjugation with an Aminoallyl dUTP incorporated during amplification. A total of 5 ug (10ug for FFPE samples) of amplified, labeled RNA was hybridized to a custom 22,000 gene microarray (Agilent, CA). This array was designed to accommodate both Fresh and FFPE samples, and contains a probe set biased for the 3' region of the RNA transcript.

Results: Presented are the relative expression levels of human cytokeratin and cytokeratin associated mRNA in adenocarcinoma of the breast and a comparison of their expression across over 60 tumor cancer types. Results will be presented which compare the expression and range of expression of these genes and their utility in differentiating site of origin.

Conclusions: Immunohistochemical studies are often used in cases of poorly differentiated tumors to resolve the site of origin. While the expression of specific proteins is often used to identify the site of origin, the distribution and range of protein expression is often poorly understood. These studies are an attempt to examine the range and distribution of gene expression across a broad range of tumors.

208 pAkt Expression Is Superior to HER-2/*neu* in Predicting Nodal Status in Breast Cancer

RStJ Ricketts, S Marconi, CN Otis. Baystate Medical Center/Tufts University School of Medicine, Springfield, MA.

Background: The PI3K/pAkt pathway is deregulated in breast cancer. pAkt plays a pivotal role in proliferative and anti-apoptotic signaling pathways. Upregulation of the PI3K/pAkt pathway is thought to impart tumors with a more aggressive behavior and a worse patient outcome. It is hypothesized that pAkt is superior to HER-2/neu in predicting nodal status in infiltrating breast carcinoma.

Design: The immunohistochemical expression of pAkt was analysed in 75 infiltrating breast utilizing an antibody to pAkt directed to serine 473 (Cell Signaling technology Beverly, MA.). The IHC analysis was performed following standard protocols on a DAKO automated platform. Tissue microarray (TMA) technology was used. Two TMAs were created; one array of 46 node negative specimens and another consisting of 29 node positive specimens. The HER-2/neu status were previously performed on these specimens (using CB11 antibody). All the specimens were formalin-fixed paraffin embedded (FFPE) tissue retrieved from the surgical pathology archives of Baystate Medical Center Department of Pathology.

Results:

HER-2/*neu* and pAkt expression in node negative cases

HER-2/*neu* expression & Nodal Status	pAkt positive	pAkt equivocal	pAkt negative
1+Node negative	0% (0 of 8 cases)	0% (0 of 8 cases	100% (8 of 8 cases)
2+Node negative	9.5% (2 of 21* cases)	4.7% (1 of 21 cases)	85.7% (18 of 21 cases)
3+Node negative	60% (9 of 15 cases)	13.3% (2 of 15 cases)	26.6% (4 of 15 cases)

*2 cases were not represented on the TMA

HER-2/*neu* and pAkt expression in node positive cases

HER-2/*neu* expression & Nodal Status	pAkt positive	pAkt equivocal	pAkt negative
1+ Node positive	77.7% (7 of 9 cases)	11.1% (1 of 9 cases)	11.1% (1 of 9 cases)
2+ Node positive	66.6% (8 of 12 cases)	16.6% (2 of 12 cases)	16.6% (2 of 12 cases)
3+ Node positive	100% (8 of 8 cases)	0% (0 of 8 cases)	0% (0 of 8 cases)

Of the 44 node negative cases represented on the TMA, 68.2% (30 of 44) cases were pAkt negative, while only 18.2% (8 of 44) of node negative cases did not demonstrate HER-2/*neu* overexpression. Conversely, pAkt was expressed in 79.3% (23 of 29) of node positive cases, while HER-2/neu overexpression was present in 55.1% (16 of 29) of node positive cases.

Conclusions: pAkt expression is superior to HER-2/*neu* in predicting nodal status in infiltrating breast cancer.

209 Role of DMBT1 (the Gene Delated in Malignant Brain Tumor 1) in Atypical Epithelial Proliferatons, In-Situ and Infiltrating Breast Carcinomas

RStJ Ricketts, S Marconi, E Dickinson, J Jerry, J Mollenhauer, AM Poustka, CN Otis, QJ Cao. Baystate Medical Center/Tufts University School of Medicine, Springfield, MA; University of Massachusetts, Amherst, MA; German Cancer Research Center, Heildelberg, Germany.

Background: DMBT1, a gene localized at 10q25.3-q26.1, is a recently identified member of the scavenger receptor cysteine rich (SRCR) super family. The SRCR proteins have been implicated in mucosal immune defense, activation of natural killer cells and tumor suppression. DMBT1 is considered as a tumor suppressor gene in glioblastoma multiforme, lung and gastrointestinal carcinomas, etc. The genetic studies and cDNA microarray analysis in breast cancer mouse model indicate DMBT1 may play a role as a susceptibility gene in breast cancer. Our recent immunohistochemical study of DMBT1 in benign breast tissue in patients without history of breast cancer vs. in patients with cancer revealed a significant decrease of its expression in patients with cancer (70.4% to 23.9%). To further elucidate the role of DMBT1 in breast carcinogenesis the expression of DMBT1 in atypical epithelial proliferations, in-situ and infiltrating carcinomas of the breast is studied.

Design: DMBT1 expression was evaluated in formalin fixed paraffin embedded tissue, including 14 specimens with atypical epithelial proliferations (11 ADH, 2 ADH/ALH, 1 ALH), 49 cases of in-situ (DCIS) and 61 cases of infiltrating carcinomas (54 infiltrating ductal carcinomas and 7 infiltrating lobular carcinomas). DMBT1 protein expression was evaluated with a monoclonal antibody utilizing standard protocols on a DAKO automated platform.

Results: Expression of DMBT1 was considered as positive only if >5% of glandular epithelium with at least moderate staining. DMBT1 expression was positive in 14.3% (2 of 14) cases with atypical epithelial proliferations, 10.2% (5 of 49) cases of DCIS and 7.9% (6 out of 76) cases of infiltrating carcinoma, respectively, compared to 70.4 % (38 of 54, prior study) benign breast tissue in patients without history of breast cancer and 23.9% (11 of 46, prior study) benign breast tissue in patients with breast cancer ($p<0.05$).

Conclusions: DMBT1 expression is significantly decreased in atypical epithelial proliferations (ADH and ALH), DCIS and infiltrating breast carcinomas, compared to benign breast tissue. These findings plus our recent genetic studies and cDNA microarray analysis indicate that DMBT1 may play a significant role as a tumor susceptibility gene in breast cancer.

210 The Use of D2-40, a Marker for Vascular Endothelium, in Diagnostic Breast Pathology

M Rivera, SL Merlin, X Chen, JM Shamonki, SA Hoda. NYPH-Weill Cornell Medical Center, New York, NY.

Background: The use of D2-40, a purportedly selective immunohistochemical marker for lymphatic endothelium, in diagnostic breast pathology remains insufficiently characterized.

Design: 30 archived cases of breast carcinoma were selected for study. One section from each case was double-immunostained for D2-40(1:50, Signet,Dedham, MA, with brown chromogen) and CD34(1:50,Biogenex, San Ramon, CA, with red chromogen). Criteria for selection of sections included presence of benign glands as well as *in situ and invasive* carcinoma. CD34 was used to mark vascular channels. For further evaluation, the chromogens were reversed.

Results: D2-40 stained endothelial cells in vessels morphologically consistent with lymphatic channels (LC) in 30/30 (100%) cases. Presence of tumor in lymphatic channels (TLC) was confirmed in 10/30 (33%) cases. (TLC had not been previously confirmed on H&E sections in 6 of these 10 cases). Assessment of TLC versus "retraction artefact" was facilitated by D2-40 in 8/30 (27%) cases. Morphometric analyses showed that the LC were relatively more numerous *vis a vis* vascular channels (VC) in the immediate vicinity (<1mm) of benign glands (mean LC:VC ratio of 3:7), *in situ* carcinoma (mean LC:VC ratio of 4:7) and invasive carcinoma (mean LC:VC ratio 3:7). LC were relatively less numerous in intramammary fibroadipose tissue (mean LC:VC ratio of 1:10), and were relatively sparse within the invasive tumor (<1:11). Myoepithelial cells were reactive with D2-40 in 1/30 cases-limiting its diagnostic uitility in that case.

Conclusions: The uses of D2-40 in breast pathology include diagnostic confirmation of lymphatic channel invasion and exclusion of "retraction artifact". The relative paucity of lymphatic channels within invasive carcinoma deserves further study.

211 Electrosurgical En Bloc® Stereotactic/Percutaneous Breast Biopsy, an Accurate Effective Method of Diagnosis

LW Rogers, SG Ries, A Sie. Long Beach Memorial Breast Center & Todd Cancer Center, Long Beach, CA.

Background: Accurate biopsy diagnosis is essential to planning definitive therapy for breast cancer and can decrease need for further, potentially more extensive surgery for benign conditions. It has been shown that larger core biopsy sizes (16ga. vs 14ga. vs. 11ga.) provide more accurate diagnoses with fewer "upgrades" than smaller cores. The en bloc® biopsy device using standard stereotactic guidance equipment, produces a single spheroid specimen measuring approximately 10x10x14mm. (10mm), or 15x15x22mm. (15mm).

Design: 144 consecutive en bloc biopsies performed in 2003-2004 were reviewed for distribution of diagnoses rendered, quality of histology (especially thermal artifact), correlation with mammogram including identification of targeted calcifications, and number of "upgrades" identified when subsequent open excisional biopsies were performed.

Results: Three initial H&E slides from each biopsy were prepared compared to an average of 12-16 slides of 11gauge core biopsies obtained for calcifications. Biopsies were performed for the following indications: calcifications (60%), mass (35%), architectural distortion (5%). Targeted calcifications were identified histologically in all cases.

Diagnoses rendered as follows: fibroadenoma (21.5%), papilloma (2%), other benign NOS(49.3%), atypical hyperplasia (6.9%), ductal carcinoma in situ (DCIS) (9.7%), invasive carcinoma (10.4%),

Thermal damage was judged minimal in most cases, but was moderate in 3 cases. In these 3 cases, operator effect appeared likely (training curve) In no biopsy did thermal artefact significantly compromise interpretation. There were 3 "upgrades" from DCIS to invasive carcinoma. In each, the DCIS was intermediate or high grade and was greater than 5 cm. in diameter. There were no "upgrades" from "atypical" to DCIS at excision.

Conclusions: The electrosurgical en bloc® biopsy method provides a superior specimen with high quality histology allowing accurate diagnosis of mammographically detected breast lesions.

212 Cell Signaling Phospho-Proteins as a Targets of HER2/neu Activity and Prognosis in BreastCancer

F Rojo, L Najera, JL Lirola, S Rodriguez, J Jimenez, MD Sabadell, J Baselga, S Ramon y Cajal. Vall d'Hebron University Hospital, Barcelona, Spain.

Background: Activation of the PI3K/Akt/mTOR signal transduction pathway is mainly dependent of membrane receptors and contributes to the development and progression of tumors by prevention of apoptosis and deregulation of cell cycle in a broad spectrum of human tumors. mTOR controls the mammalian translation machinery via activation of the p70S6K protein kinase and via inhibition of the eIF4E inhibitor 4E-BP1, and constitutes a main controller in cell growth. mTOR downstream protein 4EBP1 plays a crucial role in regulating translation and progression in cell cycle by control of cyclin D1 and c-myc.

Design: We have analyzed 56 paraffin-embedded human breast tumors with a complete immunohistochemistry profile including multiple membrane receptors and phosphorylated (p) signaling proteins: HER2/neu, EGFR, pp42/44MAPK, pAkt, p4EBP1, pp70S6K and pS6. The levels of expression were evaluated as percentage and intensity of stained tumor cells (Hscore). In parallel, a subset of frozen breast tumors was assayed by Western blotting for same markers in order to validate antibodies.

Results: Activation of PI3K/Akt/mTOR signaling cascade was significantly detected in a high proportion of breast tumors (39.48%). Patients with HER2/neu overexpression showed a higher activation of Akt compared to negative (p=0.024) and predominantly in poor differentiated tumors (p=0.029). Additionally, levels of pAkt were correlated with its downstream molecules p4EBP1 (p=0.001) and pp70S6K (p=0.05). Interestingly, p4EBP1 was mainly expressed in poor differentiated tumors (p=0.040) and significantly correlated with tumor size (p=0.018). Poor differentiation and high activation of both p42/44MAPK and 4EBP1 pathways were demonstrated in tumors with co-expression of EGFR and HER2 receptors (p=0.006).

Conclusions: Detection of activated signaling proteins by immunohistochemistry in paraffin-embedded tumors is feasible. Overexpression of erbB receptors in breast cancer induces an activation of major cell signaling pathways, and evaluation of p4EBP1 and pMAPK could reflect the real oncogenic role of this erbB receptor activation. Moreover, as new agents targeting these proteins are entering in the clinic, activation of this signalling cascade in tumors might be used to establish correlations with response.

213 Heat Shock Protein Expression in Invasive Ductal Carcinoma of the Breast

TC Rubinas, S Alkan, PB Rajan. Loyola University Medical Center, Maywood, IL.

Background: Heat shock protein (HSP) is responsible for chaperoning proteins involved in cell signaling, proliferation and survival. One promising strategy for treating cancer is to specifically target signal transduction pathways that have a key role in oncogene transformation and malignancy. Previous studies revealed that HSP plays a significant role in the cell cycle and the multi-step process of carcinogenesis. One of the HSP inhibitors, 17 allylamino-17-demthoxy geldanamycin (17-AAG) is in Phase I clinical trial due to its antitumor and synergistic activity with Taxol in human breast carcinomas. In this study we investigated the expression of HSP27 and HSP90 in benign and malignant breast tissues.

Design: Twenty-five examples of Grade 3 invasive duct carcinoma were selected for this study with 25 cases of benign breast tissue as controls. Immunohistochemical stains for HSP27 and HSP90 were performed on formalin-fixed-paraffin embedded tissue sections organized on tissue microarrays, using a standard immunoperoxidase method. Two observers independently evaluated the expression and its intensity on a scale of 1-3. Student's t test is used to calculate the level of significance.

Results: The mean HSP27 staining score in benign and malignant breast tissue was 1.0 versus 2.6 respectively. The mean HSP90 staining scores in benign and malignant breast tissues was 1.0 versus 2.5 respectively. This difference in expression in benign and malignant breast tissues is statistically significant (P< .05). Only a few invasive ductal carcinomas revealed nuclear staining for HSP27 (7/25) and HSP90 (4/25). None of benign breast tissue showed nuclear staining for HSP27 (0/25) and HSP90 (0/25).

Conclusions: Both HSP27 and HSP90 are over expressed in Grade 3 invasive breast carcinomas. HSP is expressed at a low level in benign breast tissue. These preliminary results suggest that there appears to be a significant difference in expression of HSPs in breast carcinoma supporting potential usage of HSP inhibitors in the treatment of breast cancer as evidenced by the ongoing Phase 1 clinical trial.

214 HER 2 Protein Overexpression and Gene Amplification in Breast Cancer: A Comparison of Immunohistochemistry and Fluorescent and Chromogenic In Situ Hybridization

A Sáez, FJ Andreu, S Fernández, MA Seguí, M Rey. UDIAT-Centre Diagnòstic - Corporació Parc Taulí, Sabadell, Barcelona, Spain; Corporació Parc Taulí, Sabadell, Barcelona, Spain.

Background: Objectives: 1) Establish interobserver agreement in scoring immunohistochemical results of HER-2/neu expression detected by HercepTest and CB11; 2) Correlate results of Her-2/neu expression detected with these two antibodies and the gene amplification determined by FISH; 3) Establish interobserver agreement in assessing the gene amplification by CISH; 4) Correlate gene amplification results by CISH and FISH (gold-standard).

Design: 200 cases of invasive breast carcinoma diagnosed between 2000-2003. All cases with CB11 score 2+ (43 cases) and 3+ (62 cases) and 95 random cases of score 0+ and 1+ were included. 1) Immunohistochemistry with CB11 and HercepTest. Independent observation by 2 pathologists, using the DAKO scoring system. 2) CISH technique (HER2-DNA probe). Amplification (A): >10 hybridization copies per nucleus in >50% of cells; low amplification (LA) 6-10 copies per nucleus; no amplification (NA):1-5 copies (possible polysomy, between 3-5 copies). 3) FISH technique (PathVision). Amplification: ratio of HER2/neu and chromosome 17 centromere signal counts. Ratios ≥ 2: amplification; Chr 17 polysomy: average ≥ 3 centromere signals in 60 cells.

Results: 1) Interobserver agreement (IA) for CB11 and HercepTest (84% and 86%, with k .78 and .81). IA grouping (0+, 1+) and (2+,3+) for CB11 and HercepTest (94% and 95%, with k .88 and .90). IA grouping (0+,1+,2+) and (3+) for CB11 and HercepTest (93% and 97.5%, with k .83 and .93). 2) Sensitivity CB11/HercepTest (95.2%/95.2%); specificity (70.7%/81.2%); positive predictive value (PPV) (49.3%/60.6%) and negative (NPV) (98%/98.2%). False negatives (0+, 1+): 1.8% for both antibodies. CB11 score 2+: 100% NA (15% Polysomies), score 3+: 28% NA (10.7% Polysomies). HercepTest score 2+: 94% NA (13% Polysomies); score 3+: 23.5% NA (13.7% Polysomies). 3) Interobserver agreement for CISH 93% (k .89). 4) Diagnostic accuracy for CISH: sensitivity 97.5%, specificity 94%, PPV 82.9% and NPV 99.2%.

Conclusions: 1) Excellent interobserver agreement for CB11 and HercepTest. 2) Both antibodies are valid as screening proofs with high sensitivity. 3) Score results 2+ and 3+ require determination of gene amplification. 4) CISH is a highly precise gene amplification technique with an excellent interobserver agreement. 5) CISH can be considered the most advisable technique for its lower infrastructure requirements and cost compared with FISH.

215 Distinction of Ductal Carcinoma *In Situ* from Invasive Mammary Carcinoma by MALDI-MS

ME Sanders, BJ Xu, B Shakhtour, RM Caprioli, RA Jensen, BK McLaren, DL Page, CL Arteaga, Y Shyr. Vanderbilt University Medical Center, Nashville, TN.

Background: Complex interactions among proteins in neoplastic mammary epithelium must promote breast cancer development and progression. Proteomics based discovery complements the genome initiatives and has the potential to further address the global changes controlling the heterogeneous biology of breast cancer.

Design: Proteomic spectra were obtained by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) combined with laser capture microdissection (LCM) directly from frozen sections of breast tumors (n = 122) and normal breast tissues (n = 88). First, we built a class prediction model based on differentially expressed peaks within a training cohort composed of half the tumor and normal samples (61 IMC&DCIS vs. 44 normal) which could subsequently classify a test cohort of the remaining specimens into different classes and then estimated the misclassification rate. Second, we used the leave one-out cross-validation class prediction method to estimate the misclassification rate within a test cohort composed only of the tumors (30 DCIS vs. 92 IMC). Relationships among these proteomic patterns were also examined by hierarchical clustering.

Results: More than 1600 signals were obtained collectively from the microdissected samples. Based on 25 differentially expressed peaks, a class prediction model classified tumors (IMC&DCIS) vs. normal with 95% accuracy in the training cohort and 90% accuracy in the test cohort. When the number of discriminating proteins was increased to 100, accuracy was 95% in the training cohort and 91% in the test cohort. Using 100 and 245 discriminating proteins, the leave one-out cross-validation class prediction method distinguished DCIS from IMC with 80% and 93% accuracy, respectively. Hierarchical clustering analysis showed tumor vs. normal and DCIS vs. IMC samples to cluster at roughly opposite sides of the dendrograms based on 50 and 29 differentially expressed peaks, respectively.

Conclusions: Preliminary analysis demonstrates proteomic patterns from microdissected human breast specimens can distinguish DCIS from IMC with remarkably high accuracy. Subsequent identification of these proteins should result in discovery of pathways previously unknown to play a role in breast cancer, new tumor specific markers and invasion-related proteins.

216 Focal Loss of Claudin 7 Expression in Florid Hyperplasia without Atypia

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

Background: Tight junctions maintain both concentration gradients across cell membranes and cell polarity. Claudin 7, a transmembrane protein critical to tight junctions, maintains cell-cell adhesion among epithelial cells. Recently Kominsky et al. *Oncogene 22:2021-2033, 2003* demonstrated an inverse correlation between claudin 7 expression and histological grade in both ductal carcinoma in situ (DCIS; $P < 0.001$) and invasive mammary carcinoma (IMC; $P = 0.03$). While the presence of florid hyperplasia without atypia (FHWA) in a benign breast biopsy is associated with a moderate relative risk of subsequent breast cancer, molecular markers linking subsets of at risk patients to the histologically observed architectural alterations in orientation and polarity are needed.

Design: Localization and intensity of claudin 7 expression was examined by immunohistochemistry in IMC (n =31), DCIS (n = 14), FHWA (n = 7), enlarged lobular units with columnar alteration/columnar alteration with periapical snouts and secretions (ELUCA/CAPSS; n = 3), papillary apocrine change (PAC; n = 5), and normal lobular units (NLU; n = 56). Intensity of membranous staining was graded from 0 to 3+ based on the HercepTest scoring system.

Results: ELUCA, PAC, and NLU demonstrated uniform intense basolateral 3+, 3+ and 2+ staining of luminal epithelial cells. The intensity and distribution of staining in DCIS and IMC tightly correlated with histological grade consistent with *Kominsky et al*. Basolateral 2+ staining of tubular carcinoma sharply contrasted with incomplete circumferential to absent staining in high grade IMC and DCIS. Peripherally FHWA showed 2+ basolateral staining while the characteristic centrilumenal swirling epithelial cells showed focal to complete loss of claudin 7 staining. A single focus of atypical lobular hyperplasia was characterized by 0 to 1+ expression.

Conclusions: Intense basolateral claudin 7 expression in ELUCA/CAPPS, a lesion frequently raising concern due to nuclear atypia, highlights its benignity. Aberrant claudin 7 localization and loss of expression in both FHWA and higher grade cancers reflect significant deviations from the normal orientation and polarity of mammary epithelium in these processes. Larger subsequent studies correlating claudin 7 expression in FHWA with outcome may serve to link these architectural alterations with neoplastic transformation.

217 Use of Immunohistochemistry Reduces Interobserver Variation in Classification of Papillary Breast Lesions on Core Biopsy

VI Shah, AG Douglas-Jones, M Rashid, JM Morgan, N Dallimore. Singleton Hospital, Swansea, Wales, United Kingdom; University Hospital of Wales, Cardiff, Wales, United Kingdom; Royal Gwent Hospital, Newport, Wales, United Kingdom; Llandough Hospital, Penarth, Wales, United Kingdom.

Background: This study investigates agreement on core biopsy diagnosis of papillary breast lesions, which is acknowledged as a difficult area and determines the effect of the use of immunohistochemistry to assist diagnosis.

Design: H&E sections of 129 core biopsies of breast papillary lesions were circulated to four observers who categorised each case as: B2 (benign), B3a (epithelial proliferation, probably benign but requiring biopsy), B3b (epithelial proliferation with cytological or architectural atypia), 4 (probably malignant but insufficient material or artefact to allow diagnosis), 5 (malignant papillary lesion). Immunostaining was performed for Calponin, p63 and CK5/6 and slides recirculated.

Results: There was unanimous agreement in 48% of cases on H&E only which rose to 93% after the use of immunohistochemistry. Overall unweighted Kappa (Ku; 5 categories) rose from 0.5 to 0.91 and weighted (Kw) rose from 0.76 to 0.95 after the use of IHC. The main effect of IHC was to reduce the use of intermediate categories (particularly B3a) and allow definitive diagnosis (B2 or B5).

Conclusions: Agreement on H&E sections alone in papillary core biopsies of breast is only 48% (Ku=0.50; Kw=0.76) but is significantly increased to 93% (Ku=0.91; Kw=0.95) by the use of immunohistochemistry for CK5/6, calponin and p63.

218 Benign Papillary Lesions Can Be Accurately Classified in Breast Needle Core Biopsies

VI Shah, AG Douglas-Jones, R Majid, N Dallimore. Singleton Hospital, Swansea, Wales, United Kingdom; University Hospital of Wales, Cardiff, Wales, United Kingdom; Royal Gwent Hospital, Newport, Wales, United Kingdom; Llandough Hospital, Penarth, Wales, United Kingdom.

Background: There is a general perception that benign and malignant papillary lesions of the breast cannot be reliably distinguished on needle core biopsy (NCB) specimens as a result of difficulty in interpretation of limited material and due to sampling error. There is limited data available on the histological follow-up of papillomas diagnosed on breast NCB with only a few small series published to date.

Design: 129 breast NCBs with a diagnosis of papillary lesion were identified from the histology files of three institutions during a 9-year period. The biopsies were immunostained for calponin, p63 and CK5/6. All H&E and immunostained slides were reviewed by four breast pathologists without knowledge of follow-up findings and a consensus opinion recorded as B2: benign; B3: atypical; B4: suspicious for papillary carcinoma; B5: papillary carcinoma. On follow-up, 107 (83%) of cases had undergone excision. These excision specimens were also reviewed by the four pathologists and a consensus diagnosis (B2-B5) recorded for each case. The biopsy diagnosis was correlated with that in the excision specimens.

Results:

NCB Diagnosis	Excision specimen diagnosis			
	B2	B3	B4	B5
B2 (n=39)	34 (87%)	4 (10%)	0	1 (3%)
B3 (n=10)	0	7 (70%)	0	3 (30%)
B4 (n=2)	0	0	0	2 (100%)
B5 (n=56)	0	1 (2%)	0	55 (98%)

The two cases with major discordance (NCB-B2/excision-B5 and NCB-B5/excision-B3) were both due to sampling error. The first case (radiologically R5) was related to the presence of multiple papillomas while in the second case (radiologically R3) the lesion was not represented in the excision specimen.

Conclusions: 1. Most papillary lesions can be accurately diagnosed on breast needle core biopsy. 2. Benign papillary lesions diagnosed on NCB may not require excision in the absence of suspicious clinical/radiological findings.

219 Metaplastic (Sarcomatoid) Carcinomas of the Breast Lack the Abnormally Long Telomeres of the Pleomorphic Sarcomas That They Mimic Morphologically

T Sheridan, A Meeker, J Hicks, E Montgomery, A DeMarzo, P Argani. Johns Hopkins Hospital, Baltimore, MD.

Background: Telomere length maintenance is essential for maintaining chromosomal stability and cell viability. Most carcinomas, including those of the breast (Am J Pathol 2004;164:925-35), develop short telomeres at the *in situ* stage. Despite the fact that most carcinomas reactivate telomerase to prevent tumor cell death, their telomeres remain short. In contrast, most adult pleomorphic sarcomas have dramatically elongated telomeres due to activation of the alternative lengthening of telomeres [ALT] pathway (Am J Pathol 2004;164:1523-9), and most demonstrate characteristic ALT-associated PML bodies (APB). Telomere lengths of metaplastic (sarcomatoid) carcinomas, which combine features of carcinoma and sarcoma, have not been assessed.

Design: Using a previously described fluorescence in situ method (Am J Pathol 2002;160: 1259-1266), archival tissue samples from 19 neoplasms were analyzed. The cases included 17 metaplastic carcinomas of the breast (12 grade 3, 2 grade 2, 3 grade 1) and 2 grade 3 sarcomatoid carcinomas of the bladder. Telomere signal strength (which correlates linearly with telomere length) in both the carcinomatous and sarcomatous components was compared with normal stromal cells, epithelium, and lymphocytes.

Results: The telomere lengths of the sarcomatoid components were very short in 1 case, short in 9 cases, normal in 7 cases, abnormally long in 1 case, and heterogeneous in 1 case. Importantly, ALT-associated PML bodies (APB) were not seen in any case. In 4 cases, the most pleomorphic sarcomatoid nuclei had the shortest telomeres. The telomere lengths in the carcinoma component of 12 evaluable cases were very short in 1, short in 8, normal in 2, and heterogeneous in 1. In 5 cases, the telomere length of the carcinoma component matched that of the sarcoma component, and no consistent differences were seen.

Conclusions: Unlike adult pleomorphic sarcomas, metaplastic carcinomas of the breast generally have short telomeres similar to those of usual ductal carcinomas. Therefore, while the sarcomatoid areas of these neoplasms can be morphologically indistinguishable from those of pleomorphic sarcomas, they do not acquire the ALT-associated telomere abnormalities characteristic of pleomorphic sarcomas. Different underlying genetic mechanisms may therefore lead to the sarcomatous phenotype. Similar findings in the few sarcomatoid carcinomas of the bladder studied suggests that these differences may be generalizable across different organ systems.

220 Diagnostic Utility of Cell Cycle Markers (Ki-67, skp2, p27, cyclin D1) in the Distinction between Benign and Malignant Vascular Tumors of the Breast

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

Background: Vascular tumors of the breast can pose considerable diagnostic difficulty and presently, the distinction between benign and malignant examples is made on morphologic criteria alone. Ki-67 expression has been used as an adjunct to distinguish between benign and malignant tumors at other sites. We investigated the utility of Ki-67 as a diagnostic tool as well as to better characterize the expression of other cell cycle regulatory proteins (skp2, p27, and cyclin D1) in mammary vascular tumors.

Design: 34 vascular tumors of the breast (21-hemangiomas; 13-angiosarcomas) were retrieved from our pathology files. The benign group consisted of 13 hemangiomas, 6 atypical hemangiomas, 1 angiolipoma and 1 angiomatosis. The malignant group consisted of 5 low, 3 intermediate, and 5 high-grade angiosarcomas. Immunohistochemistry was performed on 4 µm sections from a representative lesional paraffin block using monoclonal antibodies against Ki-67 (Zymed Lab. Inc.), Skp2 (clone 2C8D9, Zymed Lab. Inc.), Kip1/p27 (BD Transduction) and cyclin D1 (Lab Vision). For Ki-67, the number of positive cells was manually counted per 1000 lesional endothelial cells. For skp2, p27, and cyclin D1, a case was deemed positive if ≥10%, ≥ 50% and ≥10% of lesional endothelial cells, respectively, were immunoreactive.

Results: The mean values for Ki-67 expression (MV-Ki67) for non-atypical hemangiomas and atypical hemangiomas were 33.9 and 77.5, respectively. The MV-Ki67 for low, intermediate, and high-grade angiosarcomas were 293.8, 416.0, and 447.8, respectively. The MV-Ki67 in all hemangiomas and angiosarcomas were 46.3 and 381.2, respectively and the difference was highly significant (P<0.0001). The difference in the MV-Ki67 was also significant when atypical hemangiomas and angiosarcomas were compared (P=0.0014). Skp2 expression was greater in angiosarcomas than hemangiomas (P<0.0001) but no difference was seen with regard to p27 or cyclin D1 expression.

Conclusions: Ki-67 expression is significantly greater in angiosarcomas than in hemangiomas and atypical hemangiomas of the breast. This difference can be used as a diagnostic aid in distinguishing between these two entities. Skp2 expression was significantly greater in malignant than in benign vascular tumors of the breast; a finding to our knowledge not previously reported.

221 The Basal Subtype of Breast Carcinomas Represents the Group of Breast Carcinomas That May Benefit from EGFR-Targeted Therapies

KP Siziopikou, R Ariga, K Proussaloglou, P Gattuso, C Valadez, M Cobleigh. Rush University Medical Center, Chicago, IL.

Background: Gene array analysis of breast tumors led to the identification of tumor subtypes with potentially different biologic behavior. Targeted therapies are also increasingly successful in cancer treatment.We reported that most of the ERnegative/PR-negative/HER2 negative patients, a group that presents a therapeutic challenge, express EGFR. We now report that most of these patients belong to the basal subtype of breast tumors, and may benefit from EGFR-targeted therapies.

Design: Immunohistochemical marker profile (ER, PR, MIB-1, EGFR all Dako, CK5/6, Zymed) was obtained and analyzed by quantitative image analysis (ChromaVision, ACIS) on 271 patients diagnosed with breast cancer at Rush University Medical Center. HER2 amplification status was evaluated by FISH (PathVysion, Vysis).

Results: Overall 48/271 (18%) of tumors were positive for CK5/6 expression, thus belonging to the basal subtype of breast tumors. Most CK5/6 expressing tumors (31/48, 65%) were also positive for EGFR while only 39/223 (17%) of the CK5/6 negative tumors were EGFR positive (p<0.0001). The CK5/6 positive tumors were mostly of ductal histology (39/48). 35/48 (73%) were grade III; only 8 were grade II and one was a grade I tumor (p<0.0001). The CK5/6 positive tumors were also highly proliferative; 36/48 (75%) had unfavorable MIB-1 expression; only 73/223 (23%) of the CK5/6 negative tumors did so (p<0.0001). CK5/6 positive tumors tended to be ERnegative (35/48, 73% vs 47/223, 21%, p<0.0001) and PRnegative (75% vs 40%, p<0.0001). No strong association with patient age or tumor size was observed. Of interest, when patients were grouped by ER/PR/HER2 phenotype, 32/48 (67%) of the CK5/6 positive tumors were triple negative while only 28/223 (12%) of the CK5/6 negative patients did so (p<0.0001). Moreover, 22/32 (69%) of the basal ERneg/PRneg/HER2neg tumors were also EGFR positive while only 10/121 (8%) of the classic ERpos/PRpos/HER2neg/CK5/6neg patients expressed EGFR (p<0.0001).

Conclusions: 1. The basal subtype of breast tumors are mostly triple ER/PR/HER2 negative **2.** These lesions are high grade tumors of ductal histology with a high proliferation rate **3**. A high % of the basal tumors also express EGFR. These findings suggest that the majority of the triple negative patients have basal tumors with high EGFR expression and that this may be the group that will benefit the most from novel EGFR-targeted therapeutic strategies.

222 Detection of Chromosomal Instability Detected by Fluorescence In Situ Hybridization (FISH) in Breast Fine Needle Aspiration of Women at Risk for Breast Cancer

N Sneige, B Liu, BK Arun. U.T. M. D. Anderson Cancer Center, Houston, TX.

Background: The identification of atypia using ductal lavage or random periareolar FNA has been proposed to improve risk stratification of women who are at high risk for breast cancer. Cytologic assessment of morphologic changes, however, is subjective. A previous study suggested that chromosomal aberrations associated with breast cancer progression can be detected in cytologic specimens and that FISH may be more sensitive and specific than conventional cytology.

Design: We prospectively evaluated random periareolar FNA specimens from 30 women who were at high risk for breast cancer. In each subject, 8 FNAs were performed at the 3 and 9 o'clock positions on each breast. Cytologic specimens were prepared using the thin prep technique, and diagnoses were made on the basis of previously published criteria. One thin prep slide was evaluated by FISH using the multicolor breast aneusomy probe set for chromosomes 1-, 8-, 11-, and 17- (Vysis, Inc., Downers Grove, IL). At least 60 epithelial cells per case were scored for signal copy number. Monosomy was defined as the loss of one signal in ≥ 20% of epithelial cells, and polysomy was defined by the presence of three or more signals in ≥ 6% of cells. Cytologic smears of 5 invasive ductal carcinomas were included as positive controls.

Results: Fourteen of the 30 cases showed epithelial hyperplasia with or without atypia and the remaining 16 cases were nonproliferative. Chromosomal aberrations were detected in 14 of 16 (87%) NP cases, 11 of 14 (79%) hyperplasia cases and 5 of 5 (100%) carcinoma cases. In the high risk group, chromosomal loss was more frequently detected than chromosomal gain (64% vs. 44%, NP group; 64% vs. 43% hyperplasia group). Whereas, in the carcinoma group, chromosomal gain was the most common aberrations (100% vs. 25%). Among the four chromosomes, only chromosome 17 showed statistically significant differences in copy numbers, both gains and losses, between the high-risk and cancer groups (0.44 and 0.0002, respectively).

Conclusions: Our findings indicate that chromosomal aberrations are commonly found in women who are at high risk for breast carcinoma. No significant differences exist between the hyperplasia and NP groups that would allow categorization of equivocal cases. Whether aneusomy is a useful marker for further stratifications of women at risk for breast cancer is yet to be determined.

223 Analysis of *HER-2* Gene Amplification Using an Automated FISH Signal Enumeration System

R Stevens, I Almanaseer, M Gonzalez, D Caglar, RA Knudson, RP Ketterling, JA Bridge. University of Nebraska Medical Center, Omaha, NE; Advocate Lutheran General Hospital, Park Ridge, IL; Mayo Clinic, Rochester, MN.

Background: *HER-2* gene amplification in breast cancer is a predictor of therapeutic response to Trastuzumab (HERCEPTIN®). FISH is the current gold standard for assessment of Her-2 gene copy number. A time consuming aspect of FISH analysis is signal enumeration. In order to address this issue, Vysis has developed an automated signal enumeration system, AutoVysion™.

Design: A multi-center, blinded study was conducted on formalin-fixed, paraffin-embedded invasive breast carcinoma specimens. Hybridized PathVysion™ slides from 20 *HER-2* non-amplified breast carcinoma specimens, 19 *HER-2* amplified breast carcinoma specimens (weakly amplified to highly amplified), and associated control slides were provided in duplicate to each study site for analysis. One set of 7-8 specimen slides and 2 control slides were analyzed first by the AutoVysion™ System followed by manual enumeration within one 8-hour period. Each study site received a total of 10 slide sets to examine on separate days. Calculation of the *HER-2*/CEP17 ratio and the length of time required to analyze results by the manual and automated approaches were compared.

Results: Among all tissue specimens with informative results for both methods, overall agreement of *HER-2* classification results (positive and negative) was 92.5% (196/212). The AutoVysion™ System requires manual enumeration for cases with scanner results within the ratio range of 1.5 - 3.0. Thus, when the data in this range are excluded, the agreement between manual and scanner results is 98.8% (169/171). The average AutoVysion™ System hands-on time per slide was 4.59 minutes versus 7.47 minutes for manual signal enumeration.

Conclusions: These data support that specimens tested by the Vysis PathVysion™ assay can be correctly classified by the Vysis AutoVysion™ System and that the system may increase the overall efficiency of *HER-2* testing by reducing hands-on time in the laboratory.

224 Adenoid Cystic Carcinoma of the Breast Is Distinguished from Collagenous Spherulosis and Cribriform Ductal Carcinoma In-Situ by CD117 and Calponin Immunohistochemistry

RS Swain, C Zaloudek, DR Chase, YY Chen, JT Rabban. UCSF, San Francisco, CA; California Tumor Tissue Registry, Loma Linda, CA.

Background: Adenoid cystic carcinoma (ACC) of the breast is a rare neoplasm composed of two cell types: ductular epithelial cells and myoepithelial-like cells. The cribriform variant may be difficult to distinguish from collagenous spherulosis (CS) and low-grade cribriform ductal carcinoma in-situ (LG-DCIS), particularly in core needle biopsies. The epithelial component of ACC expresses CD117 (c-kit) but CD117 staining has not been evaluated in cribriform DCIS. The myoepithelial-like component of ACC expresses p63 and smooth muscle actin (SMA), but calponin staining has not been studied. Neither p63, calponin, nor CD117 have been evaluated in CS. We examined the diagnostic value of these markers in the differential diagnosis of these three histologically similar entities.

Design: Formalin-fixed, paraffin-embedded tissue from 8 primary breast ACC with cribriform pattern, 9 cases of CS and 4 cases of cribriform LG-DCIS were evaluated. Immunohistochemical staining for CD117 (Dako, 1:50), calponin (Dako, 1:500), p63 (Lab Vision, 1:50), and smooth muscle actin (Dako, 1:100) were performed. Membrane or cytoplasmic staining for CD117 was considered positive; nuclear staining for p63 was considered positive.

Results: While p63 and SMA marked both the myoepithelial-like cells of ACC and the myoepithelial cells of CS, calponin was negative in the myoepithelial-like cells of ACC and positive in the myoepithelial cells of CS. In cribriform DCIS, the surrounding myoepithelium expressed calponin but the neoplastic epithelial cells did not. The epithelial ductules of ACC strongly expressed CD117 while the myoepithelial-like cells were negative. Myoepithelial cells of CS were negative for CD117. Cribriform DCIS did not express CD117. Normal adjacent breast epithelium was positive for CD117 in all cases.

Conclusions: Expression of calponin by the myoepithelium of CS distinguishes it from ACC. The basal markers p63 and SMA are not helpful in this differential. Expression of CD117 by the small ductules of ACC distinguishes it from cribriform low grade DCIS and may be diagnostically helpful in problematic cases when the epithelial ductules of ACC are inconspicuous. An immunohistochemical panel that includes calponin and CD117 is useful in the differential diagnosis between ACC, CS, and cribriform DCIS.

225 Syndecan-1 and Syndecan-4 Expression in Breast Carcinoma Corrrelates with High Proliferative Rate and Absence of Estrogen Receptors

K Swartz, R Van Buren, F Baba, Y Zhang, W Wolberg, J Eickhoff, A Friedl. University of Wisconsin, Madison, WI.

Background: Syndecans are cell surface heparan sulfate proteoglycans (HSPGs) with roles in growth factor signaling and cell adhesion. We have shown that the combined expression levels of syndecan-1 (Sdc1; CD138) and syndecan-4 (Sdc4) correlate with FGF2/receptor tyrosine kinase binding (Am J Pathol. 2002, 160:185-194). The goal of this project was to investigate a possible relationship of Sdc1 and Sdc4 expression with established prognostic factors and outcome in breast carcinomas.

Design: Slides from a tissue microarray containing duplicate cores from 207 human breast carcinomas were immuno-labeled for Sdc1, Sdc4, Ki67, ER and PR and manually scored. Clinical follow-up information was available for up to 18.6 years.

Results: Sdc1 and Sdc4 expression in carcinoma cells ranged from complete loss to high expression. Both Sdc1 and Sdc4 expression were highly significantly associated with Ki67 labeling index (Sdc1: p=0.0025; Sdc4: p<0.0001) and with ER negativity (Sdc1: p<0.0001; Sdc4: p=0.0002). Both HSPGs also significantly correlated with grade, and tumor size. Sdc1 expression but not Sdc4 expression predicted patient outcome (DFS: p=0.0054; OS: p=0.0086). Multivariate analysis failed to identify Sdc1 as an independent prognostic marker, which was due to its significant association with established prognostic factors.

Conclusions: The strong association of Sdc1 and Sdc4 expression with the Ki67 labeling index suggests biologic roles in carcinoma growth regulation. The close association of these HSPGs with ER status raises the possibility of hormonal regulation. ACKNOWLEDGEMENT: This work was funded through grant BCTR0402969 from the Susan G. Komen foundation and made possible by the departmental TRIP core laboratory.

226 p53 and CD117 Immunohistochemistry in Breast Phyllodes Tumors: A Tissue Microarray Study

PH Tan, T Jayabaskar, S Selvarajan, Y Tan, MH Hilmy, G Yip, BH Bay. Singapore General Hospital, Singapore; National Kidney Foundation, Singapore; National University of Singapore, Singapore.

Background: The clinical behavior of breast phyllodes tumors (PT)is difficult to ascertain. While morphology has been used to predict clinical outcome, specific parameters defining recurrent likelihood are not agreed upon. Among recently studied biological markers, p53 and CD117 (c-kit) expression has been associated with malignant histological features. Their role in predicting recurrence is uncertain.

In this study, we investigate p53 and CD117 expression in PT in Asian women using tissue microarrays (TMA).

Design: The files of the Department of Pathology, Singapore General Hospital, were searched for PT diagnosed between January 1992 and December 2002. H&E slides were histologically reviewed, with representative areas marked for TMA construction. Using a 2 mm punch and the Beecher arrayer, donor tissue cores were transferred into recipient blocks. 4 um sections were cut from constructed TMA blocks, and subjected to immunohistochemistry for p53 (D07, Dako M7001) and CD117 (c-kit, Dako A4502) using the streptavidin-biotin method. Staining intensity and proportion of immunoreactive stromal cells were scored. Immunohistochemical results were correlated with histologic findings as well as clinical outcome; a statistically significant result was defined as p <0.05.

Results: Of 335 women diagnosed with PT, 250 (74.6%) were benign, 54 (16.1%) borderline, and 31 (9.3%) malignant. p53 and CD117, evaluated on 289 and 273 microarrays respectively, showed immunohistochemical detection in stromal cells of 105 (36.3%) cases (p53), and 17 (6.2%) cases (CD117). p53 stromal staining was associated with PT grade (p=0.004), stromal overgrowth and hypercellularity (p=0.001); while CD117 was correlated with grade (p< 0.001) stromal atypia (p =0.01), permeative margins (p =0.012), stromal overgrowth (p <0.001) and tumor size (p =0.039). Recurrences that occurred in 43 (12.8%) women revealed an association with CD117 immunostaining, but not with p53.

Conclusions: Results of this study reinforce the utility of TMA as an efficient tool to evaluate large numbers of cases. Both p53 and CD117 immunostaining can be used as adjuncts in corroborating a malignant diagnosis on histology. The association of positive CD117 stromal staining with recurrence is a useful finding, particularly in view of CD117 being a possible therapeutic target. More work needs to be done to validate the true potential of these biological markers in PT.

227 Relationship between DCIS Grade to Phenotypic Cytokeratin Markers

P Tang, Xi Wang, L Schiffhauer, P Bourne, Yi Yang, A Quinn. University of Rochester Medical Center, Rochester, NY.

Background: Breast epithelium in terminal ductal lobular unit has been subclassified based on the expression of several phenotypic cytokeratin markers as stem cell (CK5/6 +), basal cells (CK14, 17 +), and luminal cells (CK8, CK18 +), Here we describe the relationship between DCIS of different nuclear grades (non-high and high grade) and these cell differentiation markers.

Design: 99 consecutive cases of DCIS with no co-existing invasive carcinoma were retrieved from the Pathology Department files at Strong Memorial Hospital from 1997 to 2004. 53 cases of non-high grade and 46 cases of high grade DCIS were determined by consensus among three pathologists. A representative section from each case was stained with antibodies to five cytokeratin markers (stem-CK5/6, luminal-CK 8 and 18, basal-CK14 and CK17). Positive stain was defined as ≥ 10% in tumor cells.

Results: High grade DCIS shows higher expression of stem and basal cell markers than those of non-high grade DCIS (Table 1). The majority of DCIS (non-high grade and high grade) express luminal cell marker only, especially in non-high grade group. A small number of DCIS shows more complex patterns of expression, especially in high grade DCIS (Table 2), indicating other complicated alternative pathways (trans-differentiation) also exist.

Expression of each cell differentiation markers

DCIS	Stem (CK5/6)	Luminal (CK8, CK18)	Basal (CK14, CK17)
Non-high grade	7%	85%, 87%	7%, 7%
High-grade	26%	67%, 91%	24%, 26%

Expression of Cell Differentiation Markers in Each Case

DCIS	Stem (S)	Luminal (L)	Basal (B)	L+B, S+L, S+B	All (A+L+B)	None
Non-high grade	0%	87%	0%	4%	2%	7%
High Grade	2%	68%	2%	4%	22%	2%

Conclusions: 1. High grade DCIS frequently express basal or/and stem cell markers, indicating that a subset of it rises from different progenitor cells. This expression pattern in invasive carcinoma has been associated with poor prognosis. Thus, these markers may enable us to identify this more aggressive subgroup of DCIS at its earliest stage. 2. Most DCIS express luminal cell markers, suggesting that malignant transformation occurs relatively late along the cell differentiation pathway. 3. More complex carcinogenesis pathways (trans-differentiation) are frequently associated with high grade DCIS. Further studies should be done to determine its relationship with invasive carcinoma and clinical outcome.

228 Frequent Overexpression of Epidermal Growth Factor Receptor (EGFR) in Mammary High-Grade Ductal Carcinomas with Myoepithelial Differentiation (DCMD)

T Tashiro, T Shien, M Omatsu, T Masuda, K Furuta, H Tsuda, T Hasegawa. National Cancer Center Hospital and Research Institute, Tokyo, Japan; National Defense Medical College, Saitama, Japan.

Background: Approximately 2 % to 18 % of mammary ductal carcinoma are immunohistochemically positive for myoepithelial markers. We have previously described a subset of invasive ductal carcinomas (IDCs) with a large central acellular zone showing myoepithelial differentiation. Some of the most promising agents for the prevention of estrogen receptor (ER)-negative breast cancer are growth factor receptor tyrosine kinase inhibitors. The EGFR pathway contributes to a number of processes involved in tumor survival and growth, thus making it an attractive target for cancer prevention and treatment. The prognosis in EGFR+/ER- breast carcinoma patients is reportedly poor, but the histological features of breast carcinomas with EGFR overexpression remain unclear.

Design: In order to evaluate the various expressions of common biological markers and EGFR in mammary high-grade DCMD, we clinicopathologically and immunohistochemically analyzed 30 DCMD and compared these carcinomas with 36 control cases of high-grade conventional IDC.

Results: Immunohistochemically assayed expression of EGFR, HER2/neu, ER, PgR, and p53 was respectively seen in 21 (70 %), 1 (3 %), 3 (10 %), 4 (13 %), and 20 (67 %) cases of DCMD, compared with 8 (22 %), 9 (25 %), 18 (50 %), 17 (47 %), and 5 (14 %) cases of conventional IDC (P<0.05). In 16 cases (53.3 %) of DCMD, metastases were noted including brain, lung, bone, and liver within a maximum of 47 months (mean: 13.9) after the initial surgery whereas in conventional IDC there were only 4 (11%) metastatic cases to the lung and bone within a maximum of 27 months (mean: 18.0) after the initial surgery (P=0.0001). A significant difference of disease-free survival was present between DCMD and conventional IDC (P=0.001). EGFR was frequently overexpressed in DCMD compared with conventional IDC, whereas HER2/neu and hormone receptors were less expressed in the former. FISH analysis disclosed that the mean EGFR/CEP7 ratio of 24 cases of DCMD available for evaluation was 1.03, and EGFR gene amplification in DCMD was not detected in all 21 cases that had EGFR overexpression.

Conclusions: When we see suspected cases of DCMD, immunohistochemical examination for myoepithelial markers and EGFR can be useful for accurate diagnosis and molecular target therapy of this special type of IDC with a different metastatic pattern and prognosis.

229 The Value of Digital Microscopy in Enhancing Accuracy and Reliability in Grading Breast Carcinomas. The Newly Proposed KU Grading System and Its Correlation with Patients' Survival

O Tawfik, M Davis, S-M Lai, F Fan, J Donahue, P Thomas. Kansas University Medical Center, Kansas City, KS.

Background: The relationship between histologic grade and survival was first documented in the 1920's. Currently, Elston-Ellis modification of the Bloom-Richardson system (SBR) is the most widely used scheme in grading breast carcinomas. The system is hindered, however, by a lack of precision in assessing mitosis, leading to an element of subjectivity. This scheme in non-intuitive for lobular, special types or treated carcinomas and can not be used on cytology specimens. Manual mitotic count is time consuming with an inherent problem of inter- and intra-observer variability. MIB-1 proliferation index by immunohistochemistry (IHC) is a good surrogate marker for mitosis. The utilization of automated MIB-1 count can be beneficial, providing the needed precision and standardization. We previously proposed a grading system (the Kansas University [KU] system) in which we modify the SBR system by eliminating the tubular component and replacing the mitosis by an automated MIB-1 count. Our results demonstrated general agreement between the different histologic grades and all of the histologic and prognostic markers studied.

Design: 586 carcinomas were studied consisting of 156 SBR grade I, 209 grade II, and 221 grade III. The two grading systems were compared and correlated with patients' survival and histological and biomarker status, including ER, PR, p53, EGFR and Bcl-2 for 11 years duration.
Results: Utilizing the KU system we re-classify tumors from SBR grade I into KU grades I and II, SBR grade II into KU grades I, II and III, and SBG grade III into KU grades II and III. The proposed system correlated better with angiolymphatic invasion, regional lymph node metastasis, ER, PR, p53, EGFR, BCL-2, Her-2/neu status. TheKU system correlates better with patients overall survival than the SBR system. There was a significant overlap in patients' survival between SBR grades I and II. That was significantly improved by utilizing the KU system.
Conclusions: The KU grading system is superior to the SBR system because: 1) It correlates better with patients survival, 2) It is equivalent to the SBR system in its correlation with histologic grade and biomarkers status, 3) It is applicable to all epithelial carcinomas and 4) Is user friendly, computerized, and less subjective in assessing mitotic activity, therefore more reproducible.

230 Significantly Improved Sensitivity for ER Detection in Breast Cancer Using a New Rabbit Monoclonal Anti-ER Antibody (SP1)

DO Treaba, AW Hing, CC Tse, LC Goldstein, TS Barry, P Kandalaft, CB Gilks, TO Nielsen, AM Gown. PhenoPath Laboratories and IMPRIS, Seattle, WA; University of British Columbia, Vancouver, BC, Canada.
Background: Estrogen receptor (ER) expression predicts improved disease-free survival and is widely targeted in breast cancer therapy. It is therefore important to offer tests for ER by immunohistochemistry (IHC) that have the highest degree of sensitivity. In this study we compared a new rabbit monoclonal antibody (SP1) with the anti-ER mouse antibody (1D5), currently in widest use in the United States, in a large multi-institutional cohort of breast cancers to assess overall sensitivity and percentage of ER expression in individual tumors.
Design: Two different sets of specimens were employed for this study: first, a series of 119 individual breast cancers received as paraffin blocks from a wide range of institutions around the United States; second, a series of 272 breast cancer cases from the Vancouver General Hospital (VGH) represented as duplicate 0.6mm cores on tissue microarrays. All specimens were tested by IHC using 1D5 (DakoCytomation) and SP1 (Neomarkers) antibodies following optimized heat induced epitope retrieval with detection using polymer based immunoperoxidase methodology (Envision-plus, DakoCytomation.) Tissues were scored for semi-quantitative percentage of tumor cell positivity according to the following: Negative (<1%), 1+ (1-25%), 2+ (25-75%), 3+ (>75%).
Results: In the multi-institutional/whole section study, the positivity rate for the SP1 rabbit monoclonal anti-ER antibody was 84.03% compared with 78.15% for the 1D5 antibody. In the TMA based analysis, the comparable positivity rates were 78.68% for SP1 compared with 69.85% for 1D5. Overall, in the 391 specimens tested, 35/391 (8.95%) were SP1+/1D5- and 4/391 (1.02%) were SP1-/1D5+. Amongst the discordant cases, 23 (5.8%) were 2+ and 3+ with SP1 and negative with 1D5.
Conclusions: The rabbit monoclonal anti-ER antibody SP1 is significantly more sensitive compared to the mouse monoclonal antibody 1D5. This may relate to the higher affinity of rabbit monoclonal compared with mouse monoclonal antibodies, and suggests that current methods may be misclassifying a small but significant number of breast cancer patients with respect to ER status. Further studies using larger tissue microarrays will be performed to determine if classification of ER status with the SP1 antibody provides better correlation with prognosis and response to therapy.

231 Interlaboratory Comparison of ER Testing by Immunohistochemistry Using Tissue Microarrays

RR Tubbs, ED Hsi, AR Cohen, DD Dorfman, PL Fitzgibbons, MD Linden, RR Rickert, MM Bui, PC Roche, DD Murphy. College of American Pathologists, Northfield, IL.
Background: CAP Survey PM2 is an inter-laboratory comparison program for ER testing by immunohistochemistry (IHC) that utilizes Tissue Microarrays.
Design: Tissue microarrays consisting of 10 different breast cancer cores were distributed to 75 laboratories for IHC testing. Data returned by the participants included primary antibody source and type, method of antigen retrieval, and specific grading criteria. Concordance among the 75 participants was evaluated.
Results: Global agreement between all labs performing the ER TMA survey was very high, 97.5%. Results for 4 of the cores were 100% concordant among participants, 2 were 98% concordant, and 2 were 93% concordant. One core was 86.5% concordant. All cores met or exceeded the 80% concordance rate required for grading. The high concordance rate precluded evaluation of variables associated with antigen retrieval methods or antibody type.
Conclusions: A reference standard for estrogen receptor IHC assays is not available; individual responses are considered appropriate by comparing against all other participants participating in the Survey. Participant laboratories in this ER IHC TMA-based Survey performed at an exceptional level. However, this level of concordance may reflect some selection bias due to quality of the subscribing laboratories, scoring of limited amounts of tissue, and/or the inherent nature of core selection in the preparation of tissue microarrays.

232 MDM2 as an Independent Prognostic Marker in Breast Carcinoma Patients

DA Turbin, MCU Cheang, K Gelmon, E Yorida, TO Nielsen, CB Gilks, DG Huntsman. Vancouver General Hospital and University of British Columbia, Vancouver, BC, Canada; British Columbia Cancer Agency, Vancouver, BC, Canada.
Background: The MDM2 oncogene inhibits the function of the p53 tumour-suppressor protein, leading to increased cell growth, evasion of apoptosis, tolerance of genetic instability and resistance to chemotherapy. The present study was performed to evaluate the relationship of MDM2 with disease specific survival (DSS) of breast carcinoma patients.
Design: 438 breast carcinomas treated in Vancouver General Hospital in the period between 1974 and 1991 were used for building duplicate-core tissue microarrays. Formalin-fixed paraffin-embedded tissue microarray sections were immunostained with anti-MDM2 antibody (DAKO) according to standard avidin-biotin method. Slides then were digitized with a BLISS scanner, and scored semiquantitatively as negative, weak (focal strong or diffuse weak) or strong (diffuse strong) positive cytoplasmic staining. We then used an independent population-based tissue microarray validation set of 2651 cases collected from the British Columbia Cancer Agency from 1989 to 1992, to confirm the results and to perform multivariate analysis.
Results: 35 of 362 interpretable cases (9.7%) were considered to be weak positive, 14 cases (3.9%) showed strong staining, and 313 cases were negative. Kaplan-Meier analysis was performed to determine differences in DSS of the patients with negative, weak and strong expression of MDM2. There was a difference in DSS ($p=0.0022$) between the patients with tumours negative and positive for MDM2 protein (10 year DSS 72.9% vs 60.6%, resp). There was no significant difference in survival between weak positive and strong positive cases. Survival analysis of 1767 interpretable cases in the validation set confirmed cytoplasmic expression of MDM2 in 12.8% of patients and a significant difference ($p<0.0001$) in DSS between MDM2-negative and MDM2-positive cases (10 year DSS 72.5% vs 57.9%, resp). In this cohort MDM2 was an independent prognostic marker ($p=0.021$, Wald stat = 5.29) in Cox regression model with tumour grade, nodal status, ER status and tumour size.
Conclusions: Immunohistochemical studies of MDM2 in breast carcinoma on over 2000 patients show that MDM2 is independent negative prognostic marker for breast carcinoma. Even weak expression of this oncoprotein in the cytoplasm of breast tumour cells is associated with poor prognosis.

233 Syndecan-4 Is Specifically Overexpressed in Microvessels of Breast Carcinomas

R Van Buren, K Swartz, F Baba, Y Zhang, W Wolberg, J Eickhoff, A Friedl. University of Wisconsin, Madison, WI.
Background: Heparan sulfate proteoglycans (HSPGs) play an important role in angiogenesis by modulating fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) activity. Cell surface (HSPGs) are comprised mainly of the syndecan (Sdc) and glypican (Gpc) families. We have shown that in gliomas, Gpc1 is the HSPG which drives FGF2-mediated angiogenesis (J Biol Chem 2003, 278:16045). The goal of this project was to determine HSPG expression in tumor vessel endothelial cells of breast carcinomas and to correlate with outcome.
Design: Slides from a tissue microarray containing duplicate cores from 207 human breast carcinomas and normal control tissues were immuno-labeled for Sdc1, Sdc4 and Gpc1. CD31 and vWF labeling was used to identify endothelial cells. Clinical follow-up information was available for up to 18.6 years. Endothelial cell HSPG expression was scored on a scale of 0 to 3.
Results: Sdc1, Sdc4 and Gpc1 were not detectable in normal breast endothelial cells. In contrast, breast carcinoma endothelial cells showed variable HSPG expression. Endothelial cell Sdc1 was detected in 45 (22%), Sdc4 in 98 (47%) and Gpc1 in 5 (2%) cases. Thus, Sdc4 was the most prevalent endothelial cell HSPG in breast carcinomas. This prompted us to test its utility as prognostic marker. Endothelial cell Sdc4 did not correlate with pathologic stage or outcome. Surprisingly, high Sdc4 expression showed a marginally significant association with low grade ($p=0.0687$).
Conclusions: In breast carcinomas, Sdc4 is the predominant endothelial cells HSPG, which contrasts with gliomas, where Gpc1 predominates. The differential expression of different HSPGs in different tumor types suggests a distinct and specific role for HSPGs in angiogenesis. ACKNOWLEDGEMENT: This work was funded through grant BCTR0402969 from the Susan G. Komen foundation and made possible by the departmental TRIP core laboratory.

234 Histologic and Biomarker Assessment of Residual Breast Carcinoma in Surgical Specimens after Neoadjuvant Chemotherapy

RW Vancura, F Fan, C Connor, A Namiq, PA Thomas, OW Tawfik. University of Kansas Medical Center, Kansas City, KS.
Background: Neoadjuvant chemotherapy (NACT) represents the standard of care in locally advanced breast cancers. It reduces tumor volume, converts unresectable tumors to resectable, and enhances local tumor control. Complete pathological regression is not always achieved after NACT, even with apparent clinical regression. The role of the pathologist is critical in evaluating the surgical specimens to identify residual tumor. The aim of this study was to assess histologic features and the expression of prognostic markers in surgical specimens with residual cancer after NACT. These post-treatment findings were compared with the initial features of tumors before treatment. Histologic grading was performed by both the modified Scarff-Bloom-Richardson (SBR) system (combining nuclear grade, tubule formation, and mitotic count) and our own modification based on combining nuclear grade and Ki-67 count (KU system).
Design: Lumpectomy or mastectomy specimens containing residual invasive breast carcinoma after NACT from thirty patients (ages 35-64) were studied, including invasive ductal (26 cases), lobular (2 cases), and mixed ductal and lobular carcinoma

(2 cases). Nuclear grade, histologic grade (performed by two systems), and prognostic markers including ER, PR, Her-2/neu, and Ki-67 were assessed and compared with the same features of pre-treatment tumors.
Results: Residual tumor size ranged from 0.8 to 5.5 cm. Characteristic treatment effects including fibrosis, lymphocytic infiltration, and foamy histiocytes were observed adjacent to residual tumors. No statistically significant differences were found in the nuclear grade, histologic grade (by both systems), and expression of prognostic markers in residual tumors as compared to the original tumors. Performing SBR grading in residual tumors was hindered by limited residual tumor volume. The KU grading system was however easily applicable because it combined only nuclear grade and automated Ki-67 count.
Conclusions: Histologic grade and expression of prognostic markers did not change in the residual tumors after therapy as compared to the initial diagnostic tumors. Therefore, it is not necessary to repeat the measurement of markers in the residual tumor. However if grading is required for the sake of follow-up or inter-institutional comparative studies, we recommend the KU system, which is less cumbersome but has the same results as the SBR system.

235 Predicting the Status of Axillary Sentinel Lymph Nodes in 4351 Patients with Invasive Breast Carcinoma Treated in a Single Institution

G Viale, E Maiorano, G Mazzarol, G Pruneri. European Institute of Oncology and University of Milan School of Medicine, Milan, Italy; University of Bari, Bari, Italy.
Background: Reliable predictors of metastatic involvement would enable a better selection of candidate patients for sentinel lymph node biopsy (SLNB) and possibly allow identification of patients with such a low risk of axillary involvement to be even spared SLNB.
Design: We evaluated 4351 consecutive patients surgically treated for breast cancer who also underwent SLNB. Clinico-pathological features significantly associated with SLN metastases by univariate analysis were included in a multivariate model.
Results: By multivariate analysis, the prevalence of SLN metastases was directly associated with tumor size, multifocality and occurrence of peritumoral vascular invasion (PVI) (all P<0.0001), and inversely associated with favorable histotype (P=0.0007) and lack of progesterone receptors (P=0.004). A predictive model based on the features more closely associated with SLN status documented that the patients with a favorable tumor type up to 1 cm in size and without PVI (n=178, 4% of the population) had the lowest risk of SLN metastases (9.5%) whereas patients with tumors larger than 2 cm and showing PVI (n=250, 5.7% of population) had the highest risk (77.2%) of SLN involvement.
Conclusions: Tumor size and PVI emerged as the most powerful independent predictors of SLN metastases. Though no combination of features identified patients with risk of SN metastases below 9.5%, the present data may be used to tailor the management of breast cancer patients with the aim of minimizing as possible the diagnostic and therapeutic procedures thus improving the quality of life of the patients without any adverse effect on their survival rates.

236 Chromosome 10p and 12p Gains Characterise Medullary from Ductal Invasive Breast Carcinomas: A Genome Wide Array-Based Comparative Genomic Hybridization and Immunohistochemistry Analysis of 12 Cases

A Vincent-Salomon, N Gruel*, P de Crémoux, R Dendale, A Fourquet, X Sastre-Garau, O Delattre, B Sigal-Zafrani, A Aurias, * Equally contributing authors.* Institut Curie, Paris, France.
Background: Medullary breast carcinoma (MC) is characterised by a specific morphology and a biological profile with a high p53 mutation rate, ER and PR negativity. Our goal was to further genetically characterise MC by a highly resolutive genome wide array-based comparative genomic hybridisation (CGH) and to document if this specific type of breast tumor belongs immunophenotypically to the basal carcinoma group.
Design: We studied 12 MC according to Ridolfi's criteria with both a frozen sample for DNA extraction and a formalin-fixed tissue available for immunohistochemistry. ER, PR, HER2 (CB11, Novocastra), p53 (DO7, Dako), cytokeratins 5/6 and 8/18 expression were determined by immunohistochemistry on a representative tumor block. DNA profile was assessed on the BAC-PAC array with 3500 loci distributed along the genome allowing a precise delineation of the genomic imbalances with a mean resolution of 1.2 Mb. The CGH array profiles were then compared to that of a large series of 135 ductal invasive breast carcinomas previously studied.
Results: All cases were ER, PR and HER2 negative. Seven cases (58%) were p53 positive (100% of cells labeled). All cases demonstrated a CK5/6 and a CK8/18 positivity ranging from 3 to 60% and from 10 to 100% of labeled cells, respectively. Two tumors presented no genetic alteration. The DNA profile of the 10 remaining cases showed alterations identical to those observed in ductal invasive carcinomas such as gains of 1q (10 cases / 10) of 8q2.4 (8 cases /10) and of 16p (6 cases /10) and loss of the proximal 1q (10 cases/10) and of 16q (3 cases /10). In contrast, two other alterations were specifically observed in the medullary carcinomas: gains of the 10p and of the 12p in 8 of the 10 cases, those alterations being combined together, in 7/ 8 of the cases.
Conclusions: Our study confirms that MC belongs to the ER, PR, HER2 negative with a high rate of p53 overexpression group of breast carcinomas but presents a combined profile with regard to the basal and luminal cytokeratins. MC is characterized by a specific DNA profile on CGH array analysis with gains of 10p and 12p when compared to that of ductal invasive breast carcinomas. These observations confirm that MC is a specific morphological and biological entity that could deserve in a near future a specific clinical management of the patients.

237 Array-Comparative Genomic Hybridization Analysis of Infiltrating Ductal Breast Carcinoma: Identification of Amplicons Combination Related to Desease Outcome

A Vincent-Salomon, G Pierron*, V Raynal, N Gruel, S Liva, E Barillot, B Asselain, F Radvanyi, A Aurias, P Freneaux, V Dieras, F Reyal, X Sastre-Garau, JP Thiery, B Sigal-Zafrani, O Delattre, * equal contribution.* Institut Curie, Paris, France.
Background: Distinct outcomes are observed for patients with ductal breast carcinomas showing the same initial clinical presentation. Large scale analysis of the recurrent genetic alterations of the heterogeneous ductal carcinomas that could explain those differences, remains to be done.
Design: Among 135 cases of ductal carcinomas, initially treated by surgery, four groups were defined: group A (n=52): T1T2 pN0, free of disease at 80 months, group B (n=25): T1T2 pN0 experiencing metastasis within 48 months after diagnosis, group C (n=27): T1T2 T3 pN+, free of disease at 51 months and group D (n=31): T1T2T3 pN+, experiencing metastasis within 24 months after diagnosis. We have profiled DNA copy number for all tumours using a genome-wide BAC-PAC Genomic Comparative Hybridisation (CGH) array (3,500 loci distributed along the genome). The amplicons identified on the CGH profiles were then compared to the clinical characteristics of patients.
Results: Nine tumours (6%) presented no genetic alterations. 79 (52%) tumors demonstrated gains or losses of chromosomes arms only. 63 (41%) tumors presented from 1 to 3 amplicons with 17 tumors presenting only one amplified locus, 23 tumors exhibiting two locus amplified, 23 tumors presenting more than 3 amplicons. The rates of amplicons according to the groups A, B, C and D were 37%, 48%, 66% and 71% respectively. Recurrent amplifications were preferentially observed on chromosomes 17q (48 tumors), 8 (18 tumors), 11q (29 tumors), 1 (7 tumors) and 20 (14 tumors). Amplicons located at chromosomes 17 and 11 were mutually exclusive, whereas amplicons within chromosome 8 were most often associated with amplification in chromosomes 17, 11 or 1. Two amplicons located on the distal 17q (17q21.32 and 17q23.3, respectively) were also observed in addition to that of HER2 (17q12).
Conclusions: Our results show that the rate of amplicons increases in tumors associated with a poor outcome, that 17q amplicons are highly recurrent, that HER2 amplicon is associated with other amplified regions on chromosome 17q and that the amplicons involving HER2 (17q12) and CyclinD1 (11q13.3) are mutually exclusive. The mapping of amplicons together with gene expression analyses should lead to the identification of driver genes that could correspond to therapeutic target.

238 Reduction of Tumor Suppressors and Elevation of Cytotoxic Cells in Myoepithelial Cell Layers of Inflammatory Breast Carcinoma: Implication for Tumor Aggressiveness

LP Wang, C Mannion, YG Man. General Hospital of Military in Beijing, Beiijing, China; Hackensack Medical Center, Hackensack, NJ; Armed Forces Institute of Pathology, Washington, DC.
Background: Inflammatory breast carcinoma (IBC) has been reported to have the most aggressive behavior. As our previous studies have revealed that [1] the biological presentation of breast tumor cells is determined by the physical and functional status of myoepithelial (ME) cell layers, and [2] focal infiltration of immunoreactive cells impacts the physical and functional status of ME cell layers (Man et al. Breast Cancer Res 5:231-241-2004; Man and Sang. Exp Cell Res, In press), this study attempted to assess whether IBC has a higher rate of ME cell alterations and infiltration of immunoreactive cells than their biologically less aggressive counterparts.
Design: Consecutive sections from IBC (n=15) and invasive cribriform and apocrine carcinomas (n=30) with co-existing normal, hyperplastic and in situ components were immunostained for several tumor suppressor proteins produced by ME cells, and a panel of markers to immunoreactive cells. The frequency of focal ME cell layer disruptions and expression of other biomarkers in non-invasive components of these lesions were statistically compared.
Results: Distinct focal ME cell layer disruptions were seen in normal, hyperplastic, and in situ components of IBC, but the frequency was not statistically different to those in cribriform and apocrine lesions. In contrast, the intensity of the immunostaining and the number of p63, Wilms' Tumor-1(WT-1), and maspin positive ME cells in IBC were significantly lower than those in cribriform and apocrine lesions. In some cases, the entire ME cell layers in some ducts or acini in IBC was devoid of p63, WT-1, and maspin expression, although they were distinct in H&E stained sections and were strongly reactive to SMA immunostaining. In addition, ME cell layers of IBC was more frequently associated with focal infiltration of CD8, CD56, mast cell tryptase, and perforin positive cells.
Conclusions: The reduction of tumor suppressor proteins (p63, WT-1, and maspin) could result in the loss of paracrine inhibitory functions of ME cells on adjacent tumor cells. The reduction of tumor suppressors might result from elevation of CD8, CD56, mast cell tryptase, and perforin positive cells, which are cytotoxic to ME cells. (Supported in part by grants DAMD17-01-1-0129 and DAMD17-01-1-0130 to Dr. Yan-gao Man from Congressionally Directed Medical Research Programs).

239 SMMHC-p63 Cocktail Improves Detection of Myoepithelial Layer in High-Grade Ductal Carcinoma In-Situ

P Wen, WL Marsh. Ohio State University Medical Center, Columbus, OH.
Background: Myoepithelial cell (MEC) immunomarkers are sometimes used to separate in-situ from invasive mammary carcinomas. However, in the case of high-grade ductal carcinoma in-situ (HGDCIS), commonly used MEC markers are often not adequately sensitive or specific in detecting the markedly attenuated and seemingly discontinuous MEC layer that is typically surrounded by intense lymphocytic infiltrate and abundant myofibroblasts. Smooth muscle myosin heavy chain (SMMHC), a cytoplasmic marker, and p63, a nuclear marker, are among the best, in this setting. In the present study we evaluated the sensitivity and specificity of the SMMHC-p63 cocktail against SMMHC and p63 in 47 cases of HGDCIS.

Design: Formalin-fixed paraffin-embedded tissue blocks from 47 cases of HGDCIS with or without associated invasive carcinoma were retrieved from the pathology archival files. Three consecutive 4-mm thick sections from a representative tissue block of each case were immunostained separately with monoclonal antibodies against SMMHC (clone SMMS-1, Dako), p63 (clone 4A4, NeoMarkers), and SMMHC-p63 cocktail. The areas with incomplete or weak MEC staining were focused upon for comparison of sensitivity. Minimum, moderate, and marked improvement of MEC detection was defined as <5%, 5-25%, and >25% more MECs stained.
Results: Areas of weak and/or incomplete MEC layer were identified in 95.7% (45/47) of HGDCIS with separate SMMHC and p63 staining. With SMMHC-p63 cocktail, however, marked, moderate, and minimum improvement in MEC detection was seen in 46.7% (22/47), 46.7% (22/47), and 6.4% (3/47) of our cases, respectively. Minimum improvement was noted in two cases (4.3%, 2/47) where SMMHC demonstrated complete MEC staining and one case (2.1%, 1/47) that had largely nonreactive MECs with all three markers. SMMS-p63 cocktail stained both MEC cytoplasm and nuclei as expected. Simultaneous cytoplasmic and nuclear staining not only accentuates MEC staining but also allows easier separation of MECs from other cell types, such as smooth muscle cells and myofibroblasts showing only cytoplasmic staining, and tumor cells with only nuclear staining.
Conclusions: The SMMHC-p63 cocktail demonstrated improved sensitivity and specificity compared with that of SMMHC or p63 alone in the setting of HGDCIS. Improved MEC detection by the cocktail may also be valuable in evaluation of other breast lesions. SMMHC-63 cocktail may also be desirable when limited breast tissue is available for immunostaining.

240 Stromal Expression Signatures Predict Outcome in Breast Carcinoma

RB West, DSA Nuyten, S Subramanian, CL Corless, BP Rubin, K Montgomery, SX Zhu, TO Nielsen, R Patel, JR Goldblum, PO Brown, M van de Vijver, M van de Rijn. Stanford University Medical Center, Palo Alto, CA; Netherlands Cancer Institute, Amsterdam, Netherlands; University of Washington Medical Center, Seattle, WA; Vancouver General Hospital, Vancouver, BC, Canada; Cleveland Clinic Foundation, Cleveland, OH; Oregon Health & Science University Cancer Institute, Portland, OR; Emory University School of Medicine, Atlanta, GA.
Background: Many soft tissue tumors recapitulate the features of normal mesenchymal cells (leiomyosarcoma, liposarcoma, gastrointestinal stromal tumor). Numerous tumors have fibroblastic features. We hypothesize that different types of fibroblastic tumors represent different subtypes or activation states of fibroblasts.
Design: We compared the gene array expression signature of two tumor types with fibroblastic features: solitary fibrous tumor and fibromatosis. The highly expressed genes from these tumors were used to cluster 295 breast carcinomas with long-term outcome data. Cell specific gene expression was verified on tissue microarrays.
Results: Fibromatosis and solitary fibrous tumor demonstrate very different gene expression profiles including genes encoding extracellular matrix proteins and growth factors. Using a gene array data set of 295 previously published breast carcinomas, we found that there is canonical expression of a subset of these genes in the breast carcinoma samples. Moreover, samples expressing solitary fibrous tumor genes had a statistically significant worse outcome than samples expressing fibromatosis genes. A tissue microarray of breast carcinomas confirmed that the expression of selected genes within the gene set is predominantly expressed in the stroma associated with breast carcinoma and not in the neoplastic epithelial cells.
Conclusions: These findings support the existence of differences in host stromal responses to invasive carcinomas, and indicate that these differences play a role in determining clinical outcome.

241 Selection and Utility of a Panel of Early Stage Breast Cancer Prognostic Molecular Markers

CM Whitehead, R Nelson, R Hudson, M Gore, T Sporn, J Hessling, R Marcelpoil, D Morel, D Malinowski, T Fischer. Tripath Oncology, Durham, NC.
Background: 20% of breast cancer patients suffer disease recurrence and/or death within 10 years. There is a current unmet medical need to identify early stage patients and appropriately upstage their treatment regime. We have developed a panel of IHC assays that identify a large percentage of these patients. This panel augments current markers to provide the physician with molecular data revealing the activity of various oncogenic pathways. The prognostic power of these assays increases upon marker quantification using the Interactive Histology Imaging System (IHIS).
Design: A pool of candidate markers overexpressed in early breast cancer, as determined by gene array analysis, and known to play critical roles in established oncogenic pathways were identified. Antibodies raised to these candidate markers were screened against tumor microarrays composed of good or bad outcome patients. Those that exhibited increased labeling on the bad vs. good tumors were further optimized on a cohort of 200 tumors with a minimum five year follow up. Slides were scored manually by pathologists and quantified by IHIS analysis. Markers that displayed potential for clinical utility, were selected for further optimization using a second, independent 200 patient chemo naive cohort with a 10 year follow up.
Results: At each stage of the selection process the list of markers was refined. The end result was an optimized IHC panel of five markers, SLPI, E2F1, PSMB9, $p21^{ras}$ and SRC. Each of these individual markers displayed prognostic utility within our two independent patient cohorts. Specificities ranged from 78 to 92 % with corresponding sensitivities ranging from 13 to 35 %. Additional utility was extracted from these markers by analyzing all possible combinations to be positive and/or negative for each patient. This resulted in a panel of four markers that identified the poor outcome patients from our chemo naïve cohort with a specificity and sensitivity of 85 and 38%, respectively. Image analysis with the IHIS system increased the sensitivity of the marker panel to 62%.
Conclusions: We have developed and optimized IHC assays for a panel of molecular markers that exhibit prognostic utility in early stage lymph node negative breast cancer. These assays were optimized for manual pathologist scoring as well as image assisted analysis. This panel can aid in identifying early stage patients whose tumors harbor the molecular activity that increases their probability of early recurrence or death.

242 The Effect of Trastuzumab (Herceptin®) Treatment on Estrogen Receptor Status in Patients with Early Stage HER2 Positive Breast Cancer

AK Witkiewicz, N Pliss, M Kamma, S Schnitt, HJ Burstein, L Harris. New York University Medical School, New York, NY; Beth Israel Deaconess Medical Center; Dana-Farber Cancer Institute; Harvard Medical School, Boston, MA.
Background: The importance of determining estrogen receptor (ER) status of invasive breast cancers for treatment decisions is widely accepted. However de novo and acquired resistance to antiestrogens remains a significant clinical problem. There is some evidence that HER2 positive tumors are more likely to be tamoxifen resistant (Di Placido, Clin Can Res 2003) and that this resistance may be abrogated with the application of growth factor receptor inhibitors (Schiff et al, JNCI 2004). It has also been suggested that the overexpression of HER2 alters the subcellular distribution of ER by augmenting its nongenomic (membrane/cytoplasmic) activity (Kumar R, Nature 2002). The purpose of this study was to determine if treatment with the anti-HER2 antibody trastuzumab influences the nuclear expression of ER in HER2-positive breast cancers.
Design: 39 patients were assessable from a cohort with HER overexpressing stage II /III breast cancer that received 12 weeks of preoperative treatment with trastuzumab and chemotherapy (paclitaxel or vinorelbine) as part of prospective clinical trials. ER immunostaining (DakoCytomation, clone 1D5) was performed on representative paraffin sections of pre- and post-treatment samples following heat induced epitope retrieval. ER status was measured using the semi-quantitative Allred score, recording the percentage and intensity of stained nuclei. Cases were considered ER-positive when the Allred score was >2. All tumors were either HER2 3+ by immunohistochemistry or showed HER2 gene amplification by FISH.
Results: At baseline 22 patients (56%) were ER positive and 17 (44%) were ER negative. In 29 cases (74%), the Allred score post-treatment was identical to baseline. In 10 cases (26%), the pre- and post-treatment Allred scores differed, as follows: 1-2 point increase, n=4; 3 or more point increase, n=3; 1-2 point decrease, n=2; 3 or more point decrease, n=1. However, the qualitative ER status was unchanged in 38 of the 39 cases (97%). In one case ER status changed from positive to negative.
Conclusions: Preoperative trastuzumab in combination with chemotherapy does not qualitatively change ER expression in HER2 overexpressing stage II / III breast cancer. Minor changes in Allred score were noted, but these are of unclear biological or clinical significance.

243 Diagnosis of Columnar Cell Change with Atypia on Breast Core Biopsy: Impact of Inter-Observer Variability, Degree of Atypia and the Volume of Lesional Changes on Surgical Management

C Xu, M Chung, D Giri. Lifespan Academic Medical Center/Brown Medical School, Providence, RI.
Background: The diagnosis of columnar cell change (CCC) is being increasingly encountered on breast core biopsies. Surgical excision generally ensues after CCC with atypia (CCCA) is diagnosed. This paper studies the extent of inter-observer variability in the diagnosis of CCC and CCCA. Correlation was also done between the volume and degree of atypia in CCCA with findings on subsequent excision biopsy.
Design: One hundred forty six routine breast core biopsies including 106 diagnosed as CCC/CCCA at our institution were reviewed blind by two study pathologists. In each case the following was documented: histologic diagnosis, grade of atypia in CCC and the number and percent (%) of cores involved by CCC.
Results: Reviewing pathologists agreed with the original diagnosis of CCC in 82% of the cases. No atypia was diagnosed by reviewers in 7/30 (23%) cases originally diagnosed as CCCA. On the other hand CCCA was originally diagnosed in only 38/57 (67%) cases labeled as CCCA on review. Excisional biopsy was done on 39 cases. In none of the 7 cases with core biopsy diagnosed as CCC the excision revealed atypical changes. 9 (28.1%) of the cases with core biopsy diagnosis of CCCA showed significantly higher grade lesions (DCIS/LCIS/IDC) than those seen in the core biopsy: 2 IDC, 2 DCIS, 1DCIS+LCIS, 1DCIS + ALH, 2 LCIS and 1 LCIS + ADH. The average numbers of cores and percent tissue involved by atypical changes in cases which had higher grade lesions on excision (high risk group) was 4.2/8.2 (50%) and 9.2% respectively as against 2.2/6.5 (33%) and 6.5% respectively in cases where the excision showed no atypia (low risk group). The average grade of atypia was higher in the high risk group than in the low risk group (1.5/3 vs 1/3).
Conclusions: There is significant inter-observer variation in the assessment of atypia associated with CCC. The amount of atypical changes in the core biopsy as well as the degree of atypia appear to be different in the high versus low risk groups. These factors may help increase the accuracy in predicting the possibility of having higher grade lesions in the excision specimen.

244 Cytoplasmic Expression of SHPS-1 Correlates with Malignant Progression in Breast Carcinomas

T Yamamoto, K Oda, Y Nimura, M Hamaguchi. Aichi Prefectural Owari Hospital, Ichinomiya, Aichi, Japan; Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.

Background: SHPS-1 (also known as p84, BIT, and SIRP) is a heterophilic adhesive membrane protein involved in receptor tyrosine kinase signaling that is expressed widely in the mammalian central nervous system, immune system and other tissues. The function of SHPS-1 is to control cell migration and spreading.

We studied the SHPS-1 cellular localization and its significance in relation to clinicopathological features and prognosis.

Design: Five cell lines and 63 specimens of breast carcinomas were examined to evaluate SHPS-1 expression and cellular localization by immunohistochemistry and western blotting. The monoclonal antibodies used was SHPS-1 (rabbit polyclonal antibody, Neomarkers). The clinicopathological factors studied were age, staging, tumor type, histological grade and mitotic index(MI). Overall survival and diseases-free survival were evaluated using the Kaplan-Meier method.

Results: We detected 100-kDa proteins in tissue samples and cell lines by Western blotting analysis. In normal epithelium, there was a membranous expression. In breast carcinomas, 22 cases (35%) showed cytoplasmic positivity (c+), whereas 41cases (65%) were negative, There was no difference in age, staging, type between c (+) and negative's. There was a statistically significant correlation between SHPS-1 expression and histological grade: 21cases(95%) of c (+) were G2-G3 tumors ($P<0.05$). The MI for c(+) cases(1.86 ± 1.6) was significantly higher than that for negative's(0.71 ± 0.56). The cases with c (+) had poorer overall and diseases-free survival rates than negative's($P=0.044$ and $P=0.015$, respectively).

Conclusions: Our results suggest that cytoplasmic expression of SHPS-1could be associated with advanced carcinogenesis, and poor survival in the breast carcinomas.

245 Loss of 14-3-3 Sigma Expression Associates with Favorable Prognosis in Breast Carcinoma

B Yang, AA Roma, BJ Yoder, S Tarr, E Tso, T Choueiri, T Budd, JP Crowe, DG Hicks. Cleveland Clinic Foundation, Cleveland, OH.

Background: The 14-3-3 sigma protein is a p53-regulated G2/M inhibitor which regulates numerous signaling pathways involved in cell cycle control and DNA repair. Additionally, 14-3-3 sigma also inhibits apoptosis through interaction with pro-apoptotic proteins such as Bax and BAD. Reports indicate that the transcriptional expression of 14-3-3 sigma is silenced mainly through promoter methylation in breast cancer cells, however, the prognostic significance of this finding remains unclear. To further understand the role of 14-3-3 sigma in breast cancer, we have examined the relationship between 14-3-3 sigma expression and clinicopathologic features including clinical outcomes utilizing tissue microarrays (TMA) and immunohistochemistry (IHC).

Design: Five TMA's containing 163 well-characterized primary invasive breast cancers were assessed for 14-3-3 sigma expression via IHC and scored qualitatively. 14-3-3 immunoreactivity was compared to ER/PR status, *HER*2 status by bright field in situ hybridization (ISH), tumor size, histologic grade, nodal status and disease free and overall survival.

Results: Moderate cytoplasmic expression of 14-3-3 sigma was seen in 36 cases (22%) of breast carcinomas, including 35/132 (26.5%) of infiltrating ductal and 1/25 (4%) of infiltrating lobular carcinomas. The expression of 14-3-3 sigma correlated with HER2 gene amplification by ISH ($p=0.004$), while there was no significant association with patient age, ER, PR, grade, nodal status and clinicopathologic stage ($p>0.05$). There was a significantly shortened mean disease free survival (68.5 months vs 98.94 months) for tumors expressing 14-3-3 sigma ($p=0.001$), and a trend toward shortened mean overall survival (82.59 vs 98.94 months, $p=0.1$). The decreased disease free survival and overall survival associated with 14-3-3 expression were not independent of the HER2 status ($p=0.3$).

Conclusions: Loss of expression of 14-3-3 sigma protein was found in 78% of breast cancer, which associated with a lower incidence of recurrence and improved overall survival. Expression of 14-3-3 sigma was also highly correlated with HER2 gene amplification. Whether loss of 14-3-3 sigma sensitizes breast cancer cells to radiation and chemotherapy, as reported in colorectal carcinoma, needs to be further investigated.

246 Genomic Alterations in the Progression of Stage 2 Breast Carcinoma: An Array-Based Comparative Genomic Hybridization (A-CGH) Study

BJ Yoder, M Skacel, T Choueiri, DP Gaile, J Conroy, N Nowak, J Pettay, JP Crowe, RL Crownover, T Budd, RR Tubbs, DG Hicks. The Cleveland Clinic Foundation, Cleveland, OH; Roswell Park Cancer Institute, Buffalo, NY.

Background: Up to 10% of patients with stage 2 breast cancer will recur within 5 years of diagnosis. To date, no markers exist that can distinguish these "progressors" from long term, disease free survivors (non-progressors). A-CGH is a method used to screen for differential genomic gains or losses between two different, well-defined patient populations. Potential informative markers identified by A-CGH can subsequently be further studied in formalin-fixed, paraffin-embedded tissues by in-situ hybridization and/or immunohistochemistry.

Design: 22 stage 2 frozen breast cancer samples from 11 progressors and 11 age-matched non-progressors, with complete staging and clinical follow-up information, were studied. Extracted DNA from each case, containing greater than 80% invasive tumor, was analyzed via A-CGH (Roswell Park Cancer Institute, Buffalo, NY). Selected data was validated via in-situ hybridization, using probes to *CMYC*, *CCND*1, and *HER*2 genes.

Results: Global assessment of the total 4,877 RPCI-11 clones showed similar overall genomic instability between progressors (11.99%) and non-progressors (9.35%). Unique changes in non-progressors included losses of 2q24.1, 4q13.3, and 10q25.3, while progressors showed gains of 7p21.1. In addition, non-progressors more frequently had deletions of 11q14.1, 11q21, 11q22.22, and 11q23.1, while progressors showed more frequent gains of 8q21.11, 21q22.1, 21q22.2, and deletions of 10q25.3. Reciprocal changes were present at 18q11.2, with frequent gains in progressors and deletions in non-progressors. There was a good correlation between the in-situ hybridization data and A-CGH data for the *CMYC* (8q24; $r = 0.545$, pearson), *CCND1* (11q13; $r = 0.798$, pearson), and *HER*2 (17q21; p-value < 0.001, ANOVA) genes.

Conclusions: Preliminary data suggests that while stage 2 breast carcinoma progressors and non-progressors may have similar overall genomic instability, their genomic signatures appear to be unique. Additional study of these genomic regions in a larger patient sample will assess the value of these aberrations for prognostic assessment of breast carcinoma.

247 EnzMet GenePro, a Novel Bright Field Assay that Simultaneously Profiles *HER*2 Genotype and Phenotype, Correlates with Overall and Disease Free Survival of Breast Cancer

BJ Yoder, T Choueiri, E Downs-Kelly, M Skacel, J Hainfeld, R Powell, P Roche, T Grogan, J Pettay, T Budd, DG Hicks, RR Tubbs. The Cleveland Clinic Foundation, Cleveland, OH; Nanoprobes, Yaphank, NY; Ventana Medical Systems, Tucson, AZ.

Background: With the introduction of the *HER*2 antagonist, trastuzumab, precise detection of *HER*2 status in breast carcinoma has become critical. While several FDA approved assays exist for *HER*2 detection, EnzMet GenePro combines the high sensitivity and specificity of FISH (fluorescence in-situ hybridization) with the convenience and widespread availability of IHC (immunohistochemistry) into one fully automated dual color bright-field assay. We have previously shown that EnzMet GenePro has an excellent concordance with conventional FISH (87%), a high sensitivity (85%) and specificity (100%), and an excellent inter-observer reproducibility (93-100%). However, to date no clinical follow up data has been published in regards to EnzMet GenePro.

Design: Tissue microarrays (TMAs) containing 343 invasive breast carcinomas were constructed and linked to a database containing corresponding 5-year clinical follow up. These TMAs were evaluated for *HER*2 status via EnzMet GenePro and the results correlated. The IHC component of this assay (Ventana, CB11) was scored on a 0 - 3+ scale according to FDA guidelines. The EnzMet component (Nanoprobes) was scored semi-quantitatively as; amplified = 6 or more signals per nucleus, polysomic = 2–5 signals, or non-amplified = 2 or fewer signals.

Results: 32 of the 343 cases tested positive. Of the 343 cases, there were 65 recurrences and 28 breast cancer associated deaths over a 67-month follow up period. *HER2*-positive patients showed both a decreased disease free and overall survival compared to *HER2*-negative patients. This difference was statistically significant for disease-free survival ($p = 0.008$) and showed a trend for overall survival ($p = 0.06$) at the current follow-up interval (Kaplan-Meier analysis).

Conclusions: EnzMet GenePro *HER2* positive breast cancers show both decreased disease free- and overall survival compared to EnzMet GenePro-negative cases. In conjunction with previous studies, this assay combines the high sensitivity and specificity of FISH with the convenience of using conventional bright-filed microscopy for interpretation. Supported by NIH grants 2R42CA83618-02 and 1R43 GM64257-01.

248 Expression of Selenium-Binding Protein Is Decreased in Breast Ductal Carcinoma In-Situ and Infiltrating Ductal Carcinoma

C Zhang, P Zhang, CJ Sung, F Liu, MR Quddus, WD Lawrence, MM Steinhoff. Women and Infants Hospital, Brown Medical School, Providence, RI.

Background: Selenium inhibiting carcinogenesis under experimental conditions is probably mediated by selenium-binding protein (SBP). A recent study showed that reduced expression of SBP correlated with poor outcome in lung adenocarcinomas. No information, however, is available concerning SBP expression in mammary tissue. This study aims to examine the differences in SBP expression in benign mammary tissue and carcinoma.

Design: Five micron sections from 18 consecutive cases of breast carcinomas—nine with both ductal carcinoma in-situ (DCIS) and infiltrating ductal carcinoma (IDC), seven with only IDC, and two with only DCIS were stained with a monoclonal antibody against human SBP. The intranuclear and the cytoplasmic immunostains were scored using a four-scale (0 to 3+) system based on the percentage of cells stained and the intensity of staining. Immunostaining scores of DCIS, IDC, and adjacent benign breast epithelium (BB) were compared using ANOVA.

Results: In BB, SBP was present in a predominantly intranuclear pattern in ductal epithelial cells. The mean scores for intranuclear and cytoplamic stains were 2.72 and 1.67, respectively. Myoepithelial cells and stromal cells were immunonegative for SBP. Compared to ductal epithelial cells in BB, DCIS and IDC showed a significant decrease in intranuclear SBP staining ($p = 0.0001$); however, the cytoplasmic staining scores remained unchanged. No difference in SBP immunostain scores was seen between DCIS and IDC.

Conclusions: To our knowledge, this is the first study showing the presence, as well as differential expressions, of SBP in benign and malignant mammary epithelia. Decreased expression of intranuclear SBP in DCIS and IDC suggests that SBP is involved in some manner with mammary carcinogenesis. The contrast of intranuclear SBP immunostains between BB and DCIS or between BB and IDC may prove to be useful in the distinction between benign and malignant mammary epithelium in diagnostically difficult cases.

249 Frequency of Indeterminate HER-2/Neu Staining and Gene Amplification in Ductal and Lobular Carcinoma: A Comparative Study

L Zhang, H Alatassi, AW Martin, S Sahoo. University of Louisville, Louisville, KY.

Background: Her2/Neu protein overexpression and gene amplification is significantly more frequent in invasive ductal carcinoma compared with invasive lobular carcinoma. We evaluated both immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) on all invasive breast carcinomas to determine the frequency of indeterminate Her2/neu positive staining (2+ staining) and gene amplification by FISH.

Design: Four hundred thirty two consecutive cases of formalin-fixed, paraffin-embedded invasive breast carcinomas were tested for Hercept Test™ (DAKO) and FISH by PathVysion (VYSIS). Immunostained slides were analyzed either manually or quantitatively by the ChromaVision® ACIS® assisted quantitative image analysis system. The ACIS® scored the staining as follows: score 0-1.4, negative; 1.5-2.9, positive requiring confirmation of gene amplification by FISH; and ≥ 3.0 as positive. The authors reviewed all the cases scored between 1.5 to 2.9 by the image analysis system. An Her-2/neu:chromosome 17 signal ratio of > 2.0 indicated amplification of Her-2/neu gene by FISH.

Results: Of the 432 cases analyzed by IHC, 276 were negative (includes 48 cases 1+) (64%), 118 were 2+ (27%) and 38 were 3+ (9%). All the cases with indeterminate score computed by the image analysis were confirmed to be 2+ on review. Of the 118 cases with 2+ staining, 101 were invasive ductal and 17 were invasive lobular carcinomas. Among the 2+ ductal carcinomas, 6 were grade I (modified Bloom-Richardson), 34 were grade II, 55 grade III and 6 were metastases. The overall frequency of Her-2/neu gene amplification in indeterminate IHC cases was 22% (26 of 118); much higher in ductal carcinomas 25% (25 of 101 cases) than lobular 5% (1 of 17 cases) ($p< 0.05$). FISH was positive in 17 cases of grade III and 8 cases of grade II ductal carcinomas. None of the grade I ductal carcinomas showed gene amplification.

Conclusions: In this study, only 22% IHC 2+ positive cases showed gene amplification by FISH. Therefore, IHC is still a very good screening test for the evaluation of Her-2/neu gene amplification status. Although rare, lobular carcinomas with indeterminate IHC (2+ staining) need confirmation by FISH.

250 A Subset of Normal and Hyperplastic Appearing Human Breast Cell Clusters Exhibits Similar Immunohistochemical and Cytological Alterations to Carcinoma Cells

C Zhao, R Barner, T Vinh, C Walker, YG Man. Armed Forces Institute of Pathology, Washington, DC.

Background: Histological evaluation has been universally used as a "golden standard" for clinical diagnosis, while it alone often fails to detect malignant lesions that simulate normal or benign tissues, or to detect the precursor of malignant lesions. This study attempted to assess whether the combination of immunohistochemical and cytological evaluation may assist in the differential diagnosis and early detection of breast tumors.

Design: Consecutive sections from reduction mammoplasties (n=30) and from normal and hyperplastic appearing tissues associated with (n=50) and without (n=50) *in situ* and invasive malignant human breast tumors were immunostained for a panel of malignancy-associated markers, including p53, c-erb-B2, and BP1. Normal and hyperplastic appearing cells with the expression of these markers were subject to examination under the highest magnification of a light microscopy.

Results: None of the reduction mammoplasties showed the expression of p53, c-erb-B2, and BP1. Distinct expressions of these molecules, however, were seen in a subset of normal and hyperplastic appearing cells associated with and without, adjacent to or at a distance from, malignant tumors. These positive cells were generally distributed as clusters with a defined boundary to their adjacent counterparts without the expression of these molecules. The frequency and size of these cell clusters appeared to increase with tumor progression. A majority of these cell clusters showed distinct alterations in the nuclear-cytoplasm ratio, as well as nuclear shape, size, and polarity. Some of these clusters were even arranged as triangle-shaped edges protruding or "puncturing" into the stroma, similar to microinvasive lesions, or had a non-cohesive or "floating" appearance, spreading into tube-like structures that resemble blood vessels. These cell clusters, however, were often morphologically indistinguishable from clear-cut normal and hyperplastic cells on H & E stained sections at low magnification.

Conclusions: (1) These p53, c-erb-B2, and BP1 positive cell clusters might belong to a not yet defined malignant cell population or precursors of malignant lesions. (2) An integrated immunohistochemical and cytologic evaluation may have clinical value in the differential diagnosis and early detection.

Cardiovascular

251 Ultrastructural Evidence of Intercalated Disc Remodeling in Arrhythmogenic Right Ventricular Cardiomyopathy: An Electron Microscopy Investigation in Endomyocardial Biopsy

C Basso, M Della Barbera, M Valente, EK Wlodarska, B Bauce, A Rampazzo, A Nava, G Thiene, E Czarnowska. University of Padua Medical School, Padova, Italy; Children Memorial Hospital, Warsaw, Poland.

Background: Despite the advances in the pathology and pathogenesis of arrhythmogenic right ventricular cardiomyopathy (ARVC), the ultrastructural features have been overlooked so far. The recent discovery of gene mutations encoding intercalated disc (ID) proteins in both autosomal recessive and dominant forms prompted us to perform a transmission electron microscopy (TEM) study on endomyocardial biopsies (EMB).

Design: Twenty-one patients (10 M and 11 F, mean age 24,5±14 yrs) with an in vivo diagnosis of ARVC according to the task force criteria underwent right ventricular EMB. Familiarity was present in 8 (38%). Ten EMBs from donor hearts for cardiac transplantation served as controls. EMB samples were fixed in buffered 2.5% glutaraldehyde/osmium tetroxide, embedded in Epon 812 and observed under a Hitachi TEM. Myocyte nuclear, cytoplasmic organelles, contractile apparatus and ID, as well as interstitial abnormalities were assessed. In particular, IDs evaluated in terms of convolution index, D and nexus length (micron), D and nexus percent ID length, and D and nexus number per ID unity length (10 micron). Moreover, D internal and external plaques as well as gap size at the level of D, fascia adherens (FA) and nexus were evaluated.

Results: Extensive fibro-fatty replacement with a mean residual myocardium of 59±23% was found in all EMB samples at histology. TEM did not reveal major differences in terms of ID convolution index in ARVC vs controls. Mean D length and percent D/ID length were higher in ARVC than in controls (0,32±0,17 vs 0,14±0,02 and 10% vs 6%, respectively) whereas the D number/ID unity length was lower (3,38±1.47 vs 5,54±3,06) (all p value <0.01). In ARVC, abnormally located D were detected in 75% and pale internal plaques in 32%. Moreover, widening of both D (29,33±8,95 micron vs 21,68±3,42) and FA gap (41,49±20,36 vs 27,18±10,72) was found.

Conclusions: ARVC shows at ultrastructural level ID abnormalities consisting of decreased D number and increased D length, and D and FA widening in the absence of ID convolution changes. Genotype-phenotype correlation is warranted in order to assess differences between ARVC with and without gene mutations encoding D proteins.

252 Molecular Diagnosis of Acute Myocarditis Causing Sudden Death in Young People

C Basso, E Carturan, F Calabrese, G Thiene. University of Padua Medical School, Padova, Italy.

Background: Although myocarditis usually presents with signs of pump failure and ventricular dilatation as to lead to progressive systolic dysfunction, sudden death (SD) may be the unpredictable fatal clinical presentation in subjects with apparently normal hearts. Aim of our study was to assess the prevalence of viral myocarditis as a cause of SD in the young.

Design: In the time interval 1980-2004, 413 young people (<35 yrs of age, excluding SIDS) who suffered cardiac sudden death (SD) were investigated by a throughout postmortem gross and histologic protocol and 65 (16%) (39 male and 26 female, mean age 21.7±8.7 yrs) were due to acute myocarditis. Since 1998 molecular analysis on paraffin sections (26) or fresh tissue (4) of the myocardium have been also applied in a consecutive series of 30 SDs due to acute myocarditis to search for common cardiotropic DNA and RNA viruses. Sequencing analysis was used to characterize the viral genotype.

Results: A history of flu-like illness in the previous days was documented in 11 (37%). None of them had cardiac symptoms or signs either in the past or in the preceding days. At postmortem, the heart was grossly normal in all, the inflammatory infiltrate was either diffuse (11, 37%) or focal (19, 63%), and at immunohistochemistry was lymphocytic in 19 (67%) and polymorphous in 11 (33%). Clear-cut evidence of myocyte necrosis was present in 15 (50%). Nucleic acids extraction was adequate in 26 (87%) and 17 (65%) had evidence of viral infection: enterovirus in 13 (either isolated -9- or associated with cytomegalovirus in 2, and Epstein Barr virus or Epstein Barr virus and cytomegalovirus one each), parvovirus B19 in 2 (associated with herpes and Epstein Barr virus, respectively), cytomegalovirus and adenovirus one each. No difference was found in terms of myocyte necrosis and inflammatory infiltrate type and extent when comparing viral vs non viral myocarditis (all p=NS).

Conclusions: Acute myocarditis is a no so rare cause of arrhythmic SD in previously healthy young people with grossly normal heart and it is viral in origin in the majority of cases. Double/multiviral infection is common, a feature in keeping with the aggressive pattern of arrhythmogenic myocarditis. Although enterovirus are the most frequent (two-thirds of cases), other virus are detected highlighting the need for a comprehensive molecular pathology screening.

253 Distinctive Peripheral Blood Gene Expression Profiles in Patients Forming Nodular Endocardial Infiltrates (Quilty Lesions) Following Cardiac Transplantation

KE Chu, PG Lal, J Wohlgemuth, G Berry, M Billingham, CC Marboe. Columbia University, New York, NY; Expression Diagnostics, South San Francisco, CA; Stanford University, Stanford, CA.

Background: The origin and significance of Quilty lesions forming after cardiac allografting are unknown. The Cardiac Allograft Gene Expression Observational (CARGO) Study has studied peripheral leukocyte expression profiles after heart transplantation; expression profiles for patients forming Quilty lesions are compared with those of patients not forming Quilty lesions.

Design: From 8 centers, adult cardiac transplant recipients were consented and enrolled in an IRB approved study. Patients were followed at post-transplant visits with endomyocardial biopsy and collection of peripheral blood. Biopsies were graded by a panel of expert cardiac pathologists and the presence of Quilty lesions noted. Using RNA isolated from 145 peripheral blood samples representing 107 patients, real time PCR (QPCR) for 240 genes were performed. These genes were chosen either because they showed differential expression in microarray experiments or are known to be related to alloimmune mechanisms. The gene expression pattern from the Quilty formers was compared to non-Quilty formers within Not Rejecting (ISHLT biopsy grade <2 (NR)) and Rejecting (ISHLT biopsy grade >3A (R)) classes.

Results: 109 samples from 86 patients were classified as NR of which 23 (21%) had formed Quilty lesions in the first year post-transplantation (Q). 36 samples from 28 patients were classified as R of which 15 (41%) were Q. We found the expression of