tissue engineering over the last decade, however very little has been directed at the practicing pathologist.
Design: Human mesenchymal stem cells were grown in vitro in pellet culture (for chondrocyte differentiation) and monolayer (for osteogenic differentiation) for three weeks in chondrogenic and osteogenic media conditions. The cultures were then harvested, paraffin-embedded, and routine histology with hematoxalin and eosin staining was performed. Additional stains including saffranin-O for glycosaminoglycans and Von Kossa for calcium phosphate were performed on the engineered cartilage and bone, respectively, to highlight matrix production as a surrogate marker for differentiation. The morphology of the engineered bone was compared to that of native fetal and adult cartilage.
Results: The engineered tissue demonstrates similar morphology to native cartilage and bone by routine hematoxalin and eosin staining. Of note, the cell to matrix ratio of the engineered tissue lies between that of fetal and adult cartilage or bone. Other parameters such as mineralization activity and the formation of extracellular matrix, as demonstrated by Von Kossa and saffranin-O staining, paralleled that of native tissue.
Conclusions: In the near future human tissue engineered explants will require pathologic evaluation. The engineered cartilage and bone have a characteristic cell to matrix ratio, which lies in between that of fetal and adult native tissues. The phenotypic differences are highlighted here with the purpose of introducing surgical pathologists to the histology of a biomaterial that they may soon encounter in practice.

74 Fluorescence In-Situ Hybridization (FISH) Assay for Ewing's Sarcoma/PNET on Formalin-Fixed Paraffin-Embedded (FFPE) Tissue: A Pilot Study of Genetically Confirmed Cases

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Background: The diagnosis of malignant small round cell tumors including Ewing's sarcoma(ES)/PNET requires the aid of ancillary studies including immunohistochemical stains and, more recently, molecular confirmation. A FISH-based assay to detect diagnostic translocations has recently become available (LSI ® EWSR1 breakapart rearrangement probe) and may be applied to routinely processed FFPE tissues. We conducted this pilot study to determine the potential utility of using this FISH probe in the diagnosis of ES/PNET.
Design: Three cases of histologically, immunohistochemically and genetically confirmed ES/PNET were retrieved from the Hartford Hospital surgical pathology files from 2000-2001. Additional sections were prepared from representative paraffin blocks and were subjected to FISH analysis using the LSI ® EWSR1 probe (Vysis ®). Since this breakapart rearrangement probe hybridizes with EWSR1 (22q12) it will detect either the t(11;22) or t(21;22) translocation. Two additional cases of ES/PNET without genetic confirmation and four cases of non-ES/PNET round cell sarcomas were also probed.
Results:

	Diagnosis	IHC	PCR	FISH
1	ES/PNET	cd99,nse,vim,ck$^+$	EWS/FLI-1	positive(<10 cells present)
2	ES/PNET	cd99,nse,vim$^+$	EWS/FLI-1	positive(97% of cells)
3	ES/PNET	cd99,nse$^+$	EWS/FLI-1	positive(99% of cells)
4	ES/PNET	cd99,nse,vim$^+$	nd	positive(96% of cells)
5	ES/PNET	cd99,nse,vim$^+$	nd	positive(98% of cells)
6	PDSS[a]	cd99,nse,vim$^+$	nd	negative
7	RCS-NOS[b]	cd99,fli-1 (-)	nd	negative
8	RCS-NOS[b]	cd99,fli-1 (-)	negative	negative
9	RCL[c]	nd	nd	negative

nd=not done; a=poorly differentiated synovial sarcoma; b=round cell sarcoma, not otherwise specified; d=round cell liposarcoma.
Conclusions: Since all three genetically confirmed ES/PNET were FISH positive (as well as both of the histologically-suspect and IHC-confirmed ES/PNET), and the four cases of non-ES/PNET were FISH negative, this new LSI ® EWSR1 probe may prove potentially useful in the differential diagnosis of small round cell tumors, especially when fresh tissue is not available.

75 Cluster Analysis of Immunohistochemical Profiles in Melanoma and MPNST: Phenotypic Continuum and Diagnostic Strategy

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Background: Morphologic and immunophenotypic overlap between melanoma and MPNST can present a diagnostic challenge, particularly in tumors that are negative for HMB45 and Melan A. To test the usefulness of immunohistochemistry in the differential diagnosis, we studied a series of melanomas of varying subtypes and MPNST with a panel of melanocytic and neural markers by cluster analysis.
Design: Tissue microarrays containing 42 epithelioid melanomas (EM), 30 desmoplastic or spindled melanomas (DSM), and 26 MPNST were immunostained with nine selected antibodies. Intensity of staining was scored as negative, weak, or strong with attention to diffuseness and cellular localization. Tabulated data were analyzed with cluster analysis software which divided the tumors into statistically similar groups based on any positivity, strong or weak.
Results: Cluster analysis primarily divided the tumors into two large groups based upon HMB45 and/or Melan A reactivity. The positive group (n = 37) consisted entirely of melanomas, the majority (81%) being EM. The other group consisted almost entirely of HMB45 and Melan A negative tumors and clustered into three subgroups with the following predominant phenotypes: **1)** S100+, Nestin+, NGFR+, Clusterin- (13 DSM, 5 EM, 3 MPNST); **2)** Clusterin +, and mostly negative for S100, Nestin, and NGFR (11 MPNST, 2 EM, 0 DSM); and **3)** S100+, Nestin+, NGFR+, Clusterin+ (12 MPNST, 7 DSM, 1 EM). PGP9.5, Fascin, and Collagen IV were evenly distributed among the tumors and therefore had little effect on clustering.
Conclusions: These data reflect a continuum of immunophenotypic differentiation consisting of loss of melanocytic and gain of neural differentiation between EM at one end of the spectrum and MPNST at the other. EM is usually HMB45+, Melan A+, NGFR-, and Clusterin-, while DSM is usually HMB45-, Melan A-, NGFR+, and Clusterin-. MPNST shows two major immunophenotypes: one well-differentiated (S100+, Nestin+, NGFR+), the other less differentiated (S100-, Nestin-, NGFR-). However, unlike melanoma, the majority of both types of MPNST are Clusterin+. Although immunophenotypic overlap exists, a panel consisting of S100, Nestin, NGFR, and Clusterin appears to have diagnostic utility in distinguishing between HMB45/Melan A-negative melanoma and MPNST.

	Percentages of Strongly Positive Tumors					
	HMB45	Melan A	S100	Nestin	NGFR	Clusterin
EM	60	67	95	71	19	24
DSM	10	27	100	73	77	23
MPNST	0	0	54	35	35	85

76 Neurofibromatosis Type 1-Related Gastrointestinal Stromal Tumor: Special Emphasis on KIT and PDGFRA Mutations, Loss of 14q and 22q, and Activation of MAPK Pathway

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Background: Multiple GISTs rarely occur in the patients with neurofibromatosis type 1 (NF-1). The mutational inactivation of NF1 gene, which is present in NF-1 patients, results in the hyperactivation of Ras, leading the activation of downstream anti-apoptotic or growth signaling such as mitogen activated protein kinase (MAPK). Most of sporadic GISTs have a gain-of-functional mutation of KIT or platelet-derived growth factor receptor alpha (PDGFRA) with the activation of downstream RAS-Raf-MAPK pathway. Allelic losses of 14q and 22q are also common features in sporadic GISTs. However, molecular pathogenetic mechanisms of NF-1 related GISTs (NF-1 GISTs) remains unclear.
Design: In this study, we analyzed the c-kit and PDGFRA mutation, activation (phosphorylation) of MAPK p44/42, loss of heterozygosity (LOH) at 14q and 22q in NF-1 GIST. Thirty-one GISTs from five NF-1 patients and 10 sporadic tumors (10 patients) were examined.
Results: Most of NF-1 GISTs occured in small intestine. Neither KIT nor PDGFRA mutation was detected in 25 NF-1 GISTs. In contrast, KIT exon 11 mutations were detected in 7/10 (70%) sporadic GISTs. Immunohistochemical expression of phospho-MAPK p44/42 was more frequently found in NF-1 GISTs (21/25 cases; 84%) as compared to sporadic GISTs (6/10; 60%). Among the informative cases, LOH at 14q and 22q was seen in 7/8 (87.5%) and 5/12 (41.7%) of NF-1 GISTs, respectively. LOH at 14q and 22q was present in 4/8 (50.0%) and 7/8 (87.5%) of sporadic GISTs, respectively. These losses were observed in various sized of NF-1 GISTs, including small tumors less than 1 cm in size.
Conclusions: Our results suggest that LOH at 14q and 22q may contribute to the relatively early event in the tumorigenesis of NF-1 GIST as well as sporadic GIST. KIT and PDGFRA mutations are very rare event in NF-1 GIST. Rather, activation of Ras-MAPK pathway, which might be related to inactivation of NF1 gene, may play an important role in tumorigenesis of NF-1 GIST.

Breast

77 Chromosomal Alterations in Hyperplastic Regions in the Breast Depend upon the Presence of Coexisting Cancer

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Background: The role of ductal hyperplasia, usual (UDH) and atypical (ADH), as precursors to breast carcinoma (Ca) is still unclear. Therefore, we studied genomic abnormalities by fluorescence in situ hybridization (FISH) in 27 cases of hyperplasia (6 ADH) with coexisting Ca, and 17 (4 ADH) who never had Ca.
Design: Discrete areas of normal epithelium, UDH, ADH, and Ca were marked on sections for FISH. These were hybridized with 12 FISH probes in 4 multi-color panels, for 1p12, centromere (cen) 8, cen 11, and cen 17 (Breast Aneusomy Probe Set, Vysis/Abbott), TOP2A (17q21.2), MYC (8q24.21), MYH11 (16p13.11), CDH1 (16q22.1), cen 16, 1p36.3, 1q25, and COX2 (1q31.1). 20-40 cells were enumerated for each probe for each marked region, and percentage of cells with gain (>2 signals) or loss (<2 signals) of each locus determined. Student's t-test was used to compare the percentages of cells with copy number changes of each locus between histological categories.
Results: As expected, significant gains of nearly all loci were found in Ca versus normal ($p<.05$ for all loci except TOP2A). UDH or ADH versus normal regions showed significant gains for 1p12 ($p=.02$), cen 8 ($p<.001$), cen 11 ($p<.001$), cen 17 ($p<.001$), TOP2A ($p<.001$), MYC ($p<.001$), & MYH11 ($p=.007$). Normal regions showed no significant changes, even in the presence of coexisting Ca, but UDH and ADH showed gain of 1p12 ($p=.02$), 1q25 ($p=.05$), and COX2 ($p=.002$), and loss of cen 11 ($p<.001$), cen 17 ($p=.008$), TOP2A ($p=.004$), and MYC ($p=.02$) in the presence of coexisting Ca **compared to UDH and ADH without Ca**. The effect was greater if the coexisting Ca was located on the same section as UDH or ADH. ROC analysis for the presence of coexisting Ca by gene copy changes in UDH or ADH predicted that greater than 80% sensitivity and specificity might be achieved with several probe combinations (e.g. 1p12 and CEP 11), and greater than 85% sensitivity and specificity with the combination of 1p12, cen 11, and MYC.
Conclusions: Hyperplastic lesions in the presence of coexisting Ca are molecularly distinct from hyperplastic lesions without Ca. This finding has several implications with regard to carcinogenesis, and suggests that occult Ca might be detected by FISH analysis of hyperplastic lesions in breast biopsies. More studies on larger cohorts will be needed to verify these findings.

78 Oestrogen Receptors (α and β) in Primary and Recurrent Breast Cancer

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Background: Oestrogen receptor (ER) expression forms the basis for use of and response to hormonal therapy in breast cancer. ER α was the previously known type and is still the only one routinely tested for on which this important decision is based. Recently a new variant, ER β was identified. This has been shown to have distinct pattern of expression and possible clinical implication different from the already well characterized ER α. The aim of this study was to compare the pattern of expression of the 2 types of ER in primary and recurrent breast carcinoma and to correlate the result with the clinicopathologic features of the tumours.

Design: Thirty nine patients with recurrent breast tumour whose primary and recurrent tumours and clinical information were available on file at University College Hospital, Galway, Ireland were selected for the study. Sections were cut from formalin fixed and paraffin embeded tissue and stained for ER α and ER β using standard immunohistochemistry procedures

Results: The mean age of the patients was 52 years (range 32-80) with the disease free period ranging from 5 months to 170 months (median 28 months). The pattern of expression in the primary tumours are as follows; ER α alone 1(2.6%), ER β alone 20(51.3%), co expression 13 (33.3%) and expression of neither 5 (12.8%). There is higher concordance in expression of ER β in primary and recurrent tumour (82.1%) compare to ER α 35.7%. There was a significantly higher expression of ER α in lobular than ductal and in women over 60 years than those younger. This association was not seen with ER β expression. Tumours expressing ER α only or co expressing both ER α and ER β were of lower grade, lymph node negative and have longer disease free period than tumours expressing ER β alone or neither of the receptors.

Conclusions: ER β has a different pattern of expression from ER α and is associated with well established indices of poor prognosis. We also speculate that the response to hormonal therapy seen in about 10-15% of ER α negative tumour may be due to presence of ER β.

79 Prediction of Pathologic Response Based on Clinical-Pathologic Features in Breast Cancer with Neoadjuvant Chemotherapy

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Background: The aim of our study was to determine whether clinical and conventional histopathologic features of breast carcinoma (BC) assessed in core needle biopsy (CNB) before treatment can define the probability of response in patients who received neoadjuvant chemotherapy (NACT)

Design: We studied 117 CNB and the corresponding resection specimens of BC. Patients received NACT with Anthracycline +/- Taxanes (A+/-T)-containing protocols. Clinical response (cR) assessed by physical examination of the tumor size was classified as complete (100%), partial incomplete (>30%), minor (<30%) and progression. Tumor cellularity was assessed in H&E sections from CNB and surgical specimens as the percentage of tumor area with invasive cells, and graded according to Miller&Payne system. Pathologic factors of the tumor included histologic type and grade (Elston modification), necrosis (isolated or groups of cells), lymph-vascular invasion (LVI), and associated inflammatory reaction in the tumor (≥4 intraepithelial lymphocytes per high power field) and in the stroma. Lymph nodes (LN) were classified as negative when no changes or residual fibrosis was seen. The data was correlated with the pathologic response (pR)

Results: 77% of patients received treatment with A+T. Median age was 46 years (range 25-80), median clinical tumor size 57 mm (range15-120) and post-treatment 22 mm (range 1-150). Tumors were 88% of ductal type, 5% had nuclear grade 3, 55% necrosis, 21% LVI, inflammatory reaction in the tumor 12% and in the stroma 19%, and in 44% LN(-) including 9% with fibrosis. Tumor cellularity significantly decreased from a median of 32% (range 5-90%) to 5% (0-70%) following therapy. Complete cR was considered in 35% (40/115). pR was complete (G5) in 17% (20/117) and reducction in >90% (G4) in 20% (23/117). Tumors with complete pR were more frequently of ductal type (p=0.05), with nuclear grade 3 (p=0.002) and associated inflamatory reaction in the tumor (p=0.006) or stroma (p<0.000), and LN(-) (p=0.017). Moreover, >90% response rate was seen in tumors of patients ≤45 years (p=0.042), treated with A+T (p=0.018) and with necrosis (p=0.018)

Conclusions: Our results support that conventional clinicopathologic factors such as age, tumor type and grade, necrosis and inflammatory reaction are predictive factors of pR in patients in NACT. In addition, protocols including Taxanes improve the response rate. Supported by grant FIS 03/1411

80 Hormone Receptor and Her2/neu Status Pre and Post Neoadjuvant Chemotherapy in Breast Cancer: Should Immunohistochemical Studies Be Repeated?

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Background: Neoadjuvant chemotherapy can be used prior to surgical excision of locally advanced breast carcinomas. Immunohistochemical (IHC) markers are typically performed on core needle biopsies prior to therapy with the goal of guiding therapeutic options and establishing prognosis. However, it is unclear if these markers should be repeated on the post-treatment surgical specimen. Does neoadjuvant chemotherapy change ER, PR and Her2/neu IHC status?

Design: 120 cases of breast carcinoma diagnosed on core needle biopsy and treated with neoadjuvant chemotherapy were identified in the University of Washington pathology database. Of these cases, 45 had both residual carcinoma in the post-therapy excisions and corresponding blocks available for immunostaining. Cases were stained with ER (1D5), PR (PR88) and Her2/neu (polyclonal) antibodies. These results were compared to the IHC results from the original pre-neoadjuvant core needle biopsies.

Results: Of the 45 cases of post-neoadjuvant invasive breast carcinomas, a change in IHC results was noted in 16 cases (35%). The majority of changes were in PR status, which became negative in 11 of 28 cases previously PR positive (two of which were also ER negative). ER status only changed to negative in 1 of 29 previously positive cases. Her2/neu immunostaining was negative (0-1+ staining) in 4 of 18 previously high overexpressed cases (22%) and intermediate for over-expression (2+) in an additional 2 of these 18 cases. A single case that was negative for Her2/neu overexpression on core needle biopsy was 3+ positive (high over-expression) on the post-therapy excision. Of the 16 cases that had changes in marker status, 13 had changes in only one antibody, 2 had changes in both Her2/neu and PR status and a single case had changes in all three antibodies. Overall, 9 of the 45 cases (20%) had changes in hormone receptors or Her2/neu IHC status that could have altered therapeutic decisions about treating with hormone receptor antagonists or Herceptin.

Conclusions: Her2/neu status of breast carcinomas can change post-neoadjuvant chemotherapy. A change in hormone receptor status can also occur but is less likely to affect both ER and PR status. Repeating immunohistochemical markers on post-neoadjuvant chemotherapy specimens should be considered because the results may alter therapeutic decisions.

81 Should All Positive Immunohistochemistry (IHC) HER-2/neu over-Expression Be Tested by Fluorescent *In Situ* Hybridization (FISH) in Breast Cancer Cases Patients? 5-Year Community Based Study

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Background: Over-expression/amplification of the HER-2/neu (C-erbB-2) proto-oncogene is found in 20-30% in breast cancer patients, is associated with poor prognosis, and has major implications for adjuvant therapy. Most clinical laboratories currently use IHC for the initial assessment with reflex FISH confirmation for borderline (2+) cases. Generally, tumors with 3+ amplification status by IHC are not referred for alternative testing by FISH. However, a number of studies have reported a significant fraction of 3+ IHC that are FISH negative. We present a 5-year experience in a community based setting.

Design: 1427 invasive breast carcinomas were examined by IHC using the HercepTest® (DakoCytomation). Borderline (2+) cases were reflex tested by FISH. 83 of the 160 (51.9%) 3+ cases, with available blocks, were also tested by FISH using the PathVysion HER-2 DNA probe kit (Abbott Laboratories). Tumor type, overall tumor grade, grading components (nuclear grade, tubule formation, and mitotic activity), estrogen [ER] and progesterone receptor [PR], lymphovascular invasion (LVI), and nodal status, were recorded for all samples.

Results: HER-2neu was negative for over-expression in 1143 of 1427 cases (80.1%), positive (3+) in 160 cases (11.2%), and borderline (2+) in 124 cases (8.7%). FISH amplification was detected in 67 of 83 (80.7%) 3+IHC and in 18% of the 2+ IHC positive cases. ER and PR negative cases were associated with FISH amplification (2+ and 3+ IHC cases combined) [P=0.034 and P<0.0001 respectively]. PR negative status was a marker for 2+IHC/FISH amplification positive tumors (P=0.009). Tumor characteristics distinguishing 3+IHC/FISH amplification positive tumors from 3+ IHC/FISH negative tumors were not detected.

Conclusions: In this study, only 18% IHC 2+ positive cases showed gene amplification by FISH supporting IHC as an appropriate initial screening test for the evaluation of Her-2/neu status. The proportion of non-amplification in IHC 3+ positive cases (19.2%) is higher than expected suggesting FISH testing be considered for all IHC 3+ cases. However larger studies are required to determine whether patients with IHC 3+/FISH-negative breast tumors benefit from Herceptin treatment and to confirm there are no tumor characteristics distinguishing these tumors from IHC 3+/FISH positive tumors.

82 "Isolated Tumor Cells/pN0(I+)" Category of Sentinel Lymph Node Staging Represents a Wider Range of Tumor Involvement Than That Implied by the Term "Isolated"

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Background: Per current TNM Classification, the "Isolated Tumor Cells (ITC)/ pN0(i+)" category for sentinel lymph nodes (SLNs) are "single…or small clusters of cells, not more than 0.2 mm…that are usually detected by immunohistochemistry". Recent publications have highlighted problems with this definition (*Int J Surg Pathol* 2004;12:301-306; *Cancer* 2005;103:358-367).

Design: Retrospectively reviewed was clinicopathological material from all SLNs ('02-'05) which could be categorically staged as ITC/pN0(i+). All SLNs had been routinely processed at 3 H&E levels and by 1 CK AE1/3 immunostain.

Results: 50 SLNs from 46 patients (age:30-87, mean:56) were reviewed. Primary tumor type was ductal:34, lobular:8, other:4. One or more prior biopsy procedure had been performed in all 46 cases (including FNA:6, core:31). Lymphovascular invasion was noted in 14 (30%) cases. H&E slides of SLN were "negative", i.e. imperceptible, in 39/ 50 (78%); and equivocal (+), i.e. barely perceptible:needed CK confirmation, in 11/50 (22%). Number of CK(+) cells ranged from 1-90 (mean:16). Solitary CK(+) cell was seen in 8/50 (16%), multiple isolated single CK(+) cells in 11/50 (22%), and clusters of CK(+) cells in 31/50 (62%). CK(+) cells were located only in the subcapsular sinus in 37/50 (74%). Additional nodes were examined in 21/46 cases, with (+) nodes in 4/21 (19%); number of CK(+) cells in SLN (range:1-90, mean 18) was not predictive of additional node involvement in these cases.

Conclusions: Isolated Tumor Cells/pN0(i+) category of SLN, as defined by current TNM Classification, represents a wider range of tumor involvement than that implied

by the term "isolated". "Isolated" is misleading in this setting- as in this study, the mean number of CK(+) cells was 16, and only 16% of SLN showed truly "isolated" (per *Webster's Dictionary:* alone or solitary) cells.

83 BRCA2 Associated Breast Cancers Exhibit a Luminal Phenotype Despite their High Histologic Grade

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Background: Comprehensive molecular profiling of human breast carcinomas using cDNA expression arrays has lead to the identification of genetically distinct subgroups with different prognoses. These are luminal, normal breast-like, HER2 overexpressing and basal-like groups. BRCA1 associated hereditary breast cancers cluster closely with the basal-like group. The molecular profile of BRCA2 associated hereditary breast cancers is less certain. The objective of our study was to determine where these cancers lie within the new paradigm of breast cancer molecular classification.

Design: 157 BRCA2 associated breast cancers and 314 age and ethnically matched control tumors underwent a semi-centralized pathologic review within the International Breast Collaborative Family Registry. Of these, 64 BRCA2 associated tumors and 186 controls were available for TMA construction. TMA sections were analyzed for hormone receptors, HER2/neu protein overexpression, basal and luminal cytokeratin expression and Ki67. Odds ratios and p values were calculated for each variable.

Results: BRCA2 associated breast tumors were predominantly invasive ductal, no special type carcinomas, Nottingham grade III/III (p=0.001) with pushing tumor margins (p=0.01) and a high proliferative index (Ki67, p=0.02). By tree-view cluster analysis BRCA2 associated tumors, despite their high histologic grade, had a tendency to cluster in the luminal category as evidenced by the strong expression of ER (p=0.05) and a trend towards greater expression of the luminal cytokeratins CK8/18, low expression of the basal cytokeratin CK5, infrequent accumulation of mutant p53 and low frequency of HER2/neu overexpressing tumors

Conclusions: Despite the fact that BRCA2 associated hereditary breast tumors are predominantly poorly differentiated tumors with pushing tumor margins, these tumors have a very different molecular profile than BRCA1 associated tumors, with strong expression of ER and luminal cytokeratins. These findings may have implications for both chemoprophylaxis and prognosis.

84 The Molecular Heterogeneity of LCIS and Its Biological/Therapeutic Correlates

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Background: Lobular carcinoma in situ (LCIS) is a poorly understood breast epithelial proliferative lesion that involves terminal lobules and is thought to increase the risk of breast cancer 10 fold. Although all types of LCIS are regarded by pathologists, surgeons and oncologists in the same way as increasing breast cancer risk, the disease might not be the same disease in every patient. LCIS may be, in fact, quite a heterogeneous disease. Some types of LCIS may be innocuous, some types associated with increased risk of invasive breast cancer and some types actually clonally progress into invasive lobular carcinoma.

Design: Using a combination of laser capture microdissection and the identification of a number of different DNA microsatellite loci for either loss of heterozygosity (LOH) or gain of copy number (microsatellite instability), we studied 300 cases of LCIS retrospectively. Some of these cases (20%) had concomitant invasive ductal or (10%) invasive lobular carcinoma and some (70%) did not.

Results: In our initial studies three groups of LCIS emerged. The first type of LCIS shared a common chromosomal loss (LOH) or gain (microsatellite instability) pattern with concomitant invasive lobular carcinoma suggesting that this type of LCIS had, in fact, clonally progressed to invasive lobular carcinoma; the second type of LCIS exhibited a consistently different chromosomal loss (LOH) or gain (microsatellite instability) pattern from the concomitant invasive carcinoma that was present suggesting that this type of LCIS was, at most, only a risk factor for invasive carcinoma; the third type of LCIS showed no obvious genomic alterations (losses or gains) over 15 different microsatellite loci suggesting that this type of LCIS might be a type of hyperplasia rather than neoplasia and therefore a more innocuous lesion. This last type of LCIS was not associated with concomitant invasive carcinoma.

Conclusions: Our three-branched LCIS molecular stratification scheme, which needs to be studied prospectively, has potential therapeutic implications. If one is dealing with an LCIS that will progress to invasive cancer, the therapy should be surgical excision (analogous to the situation in DCIS). If one is dealing with an LCIS exhibiting genomic alterations not associated with direct clonal progression to invasive carcinoma, chemopreventive strategies, eg., tamoxifen, might be in order. On the other hand, if one is dealing with an LCIS type with no genomic alterations, a "wait and watch" policy might be the best medicine.

85 Histologic and Immunophenotypic Features of Estrogen Receptor, Progesterone Receptor, Her2/neu Negative Breast Carcinomas

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Background: Triple negative breast carcinomas (estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu (HER2) negative) are typically aggressive tumors that are unresponsive to many therapeutic agents used to treat conventional type carcinomas. The objectives of this study were to evaluate the expression of markers of potential prognostic and therapeutic significance in this subset of tumors, and to identify histologic features that may correlate with expression of these markers.

Design: 104 triple negative primary invasive breast carcinomas collected between 1/04 and 7/05 were incorporated into tissue microarrays (TMAs) using duplicate or triplicate 1 mm cores from each case. All tumors were evaluated for multiple histologic features including tumor type, grade, architectural growth pattern, presence or absence of intratumoral stroma and/or lymphoplasmacytic infiltrate, and circumscription. Each TMA was immunostained for EGFR, p53, c-kit, E-cadherin, CK5/6, and K903. Staining was considered positive if observed in at least 10% of tumor cells.

Results: The 104 tumors included the following histologic types: 83 grade 3 ductal carcinomas, not otherwise specified (80%); 11 metaplastic carcinomas (10%); 8 tumors with medullary-like features (8%); and 2 grade 2 ductal carcinomas (2%). EGFR was the most commonly expressed marker (57/99, 58%), followed by p53 (52/100, 52%), and c-kit (37/100, 37%). Markers of basal-like differentiation including K-903 and CK5/6 were positive in 77/95 (81%) and 40/101 (40%) tumors, respectively. 62% of K903 positive tumors were also EGFR positive. Complete loss of E-cadherin immunoreactivity was seen in 21/99 tumors (21%), and partial loss was seen in 54/99 tumors (54%). No statistically significant correlation was noted between any 2 markers or any single histologic feature and expression of any marker.

Conclusions: While the ER, PR, HER2 negative carcinomas studied were predominately grade 3, no other specific histologic feature (growth pattern, intratumoral lymphocytes and/or stroma, or circumscription) was predictive of immunophenotype. Overall, these tumors displayed striking loss of E-cadherin (75%), as well as frequent positivity for EGFR (58%); therefore incorporation of these markers in the standard prognostic/predictive panel of breast cancer should be considered.

86 The Value of Soy Isoflavones in Modulating Biomarkers of Growth and Differentiation in Breast Cancer

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Background: Epidemiologic studies suggest a role of soy in cancer prevention, and preclinical studies indicate that soy isoflavones (SI) act as potent modulators of various cellular pathways implicated in carcinogenesis. This study was undertaken to determine the effects of SI on neoplastic and adjacent normal tissue in women with breast cancer.

Design: 64 women with newly diagnosed breast carcinoma (DCIS or invasive) were randomly assigned to receive either placebo or oral SI 100 mg/day (50 mg genistein) for 3 wks before excision. All subjects had SI levels measured at baseline and after intervention. The expression of bcl-2, cyclin B1, bax, Cx43, p21 and pFAK were analyzed by western blot and those of EGFR Related Protein (ERRP), Cx43, p-AKT, p-FAK, p21, Caspase-3 and Ki-67 were analyzed by immunohistochemistry (IHC).

Results: *Western blot:* There were significant decreases in Cx43 and p21 expression in the SI group compared to placebo in both benign (p=0.0614, p=0.0026) and tumor tissues (p=0.0668, p=0.0072). Similarly, the subjects with increase in genistein concentration had lower Cx43 and p21 expression compared to placebo in benign (p=0.0387, p=0.0060) and tumor tissues (p=0.0563, p=0.0194). In addition, subjects in SI group had significantly lower bcl-2, Cyclin B1 and Bax concentrations compared to placebo in normal tissues (p=0.0079, p= 0.0079, p=0.0432) but not in tumor tissues. Similarly, the subjects with increase in genistein concentration had lower bcl-2 and Bax expression in benign tissue (p=0.0282, p=0.0433), but not in malignant tissue. This subgroup had also decreased cyclin B1 and pFAK in the entire group (p=0.0780, p=0.05) but not statistically significant in the subgroups of benign and tumor tissues. *IHC:* There were trends towards increase in ERRP (p=0.593) in malignant and benign parts of the tissue specimens in SI group but rest of the biomarkers studied showed no statistically significant changes between the groups.

Conclusions: When administered at a dose of 100mg/day, SI down-regulated various molecules including Cx43 and p21. Further investigations are needed to determine whether this down-regulation is a dose-dependent phenomenon, and whether SI may become pro-estrogenic (rather than anti-estrogenic) at certain levels and thus may promote breast cancer rather than prevent it.

87 Analysis of Cancer Risk in Women with Radial Scars of the Breast

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Background: Radial scars (RS) are characterized by an elastotic central core containing entrapped tubules that radiate outward in a stellate manner. The epithelial component may show varying degrees of proliferation. Previous studies have shown that RS impart an increased risk of breast cancer development.

Design: Radial scars were systematically identified in a histopathologically defined benign breast disease (BBD) cohort of 9073 patients biopsied between 1967 and 1991. Overall histology was classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH) per standard criteria. The presence, number, and size of RS were counted for each case. The relative risk of cancer development within the BBD cohort was compared to that expected in the general population using standardized incidence ratios (SIRs, mean follow-up interval 17 years).

Results: RS were identified in 441 (4.9%) of the cohort cases; 384 (87%) of these contained one RS, 42 (9.5%) contained two, nine (2%) contained three, and six (1.5%) contained four or more, with a maximum of 11. RS size information was available in 434 cases. The majority of RS (357/434, 82%) were less than 5mm in diameter; 61 biopsies (14%) contained from 5-9.9mm RS; and 16 (4%) had RS 10mm or greater in diameter. Tables 1 and 2 compare the relative risk of BBD subsets defined by presence, size, and number of RS to patient groups lacking RS.

Incident Breast Cancer Relative Risk

Diagnosis	Number Eligible Women	Relative Risk (95% CI)
NP	6048	1.07 (0.96, 1.18)
PDWA	2311	1.57 (1.37, 1.80)
PDWA + RS	377	1.84 (1.33, 2.49)
AH	273	4.01 (3.03, 5.21)
AH + RS	64	3.33 (1.67, 5.97)

Relative Risk By Radial Scar Feature

Feature	Number Eligible Women	Relative Risk (95% CI)
1 Scar	384	2.02 (1.48, 2.69)
2+ Scars	57	2.12 (0.85, 4.35)
Size < 5 mm	357	1.84 (1.32, 2.51)
Size > 5 mm	77	2.50 (1.20, 4.61)

Conclusions: RS imparts no increased breast cancer risk compared to other forms of PDWA (i.e. duct hyperplasia and/or adenosis). Likewise, RS associated with AH also connotes no increased risk above that of AH. Breast cancer risk was not modified significantly by the size or number of RS lesions.

88 Mammaglobin Versus GCDFP-15: An Immunohistologic Validation Survey for Sensitivity and Specificity

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Background: There is a dearth of antibodies that are useful to identify breast carcinomas. GCDFP-15 has a high specificity for breast carcinoma but has a sensitivity that is less than optimal. Mammaglobin is a 10.5 kD secretory protein that shows reactivity with normal breast epithelium. There is no data that compares the utility of mammaglobin to GCDFP-15 for sensitivity and specificity in the identification of breast carcinomas.

Design: Whole tissue sections of 58 breast carcinoma cases (14 ductal, 14 lobular and 1 mixed breast carcinomas with matched lymph node metastases) were stained with GCDFP-15 (Cell Marque, Hot Springs, AZ) and mammaglobin cocktail (Zeta Corporation Sierra Madre, CA) antibodies. In addition, tissue microarrays (US Biomax, Inc. Rockville, MD) containing 80 melanomas, 60 endometrial tumors, 41 cervical squamous cell carcinomas, 45 lung tumors, 39 stomach tumors, 39 colon tumors, 40 kidney tumors and 40 bladder tumors were also stained with mammaglobin cocktail antibody. For meaningful semi-quantitative analysis, only patchy/diffuse cytoplasmic staining with moderate or strong intensity was considered as positive staining. Focal/weak staining or no immunoreactivity was considered as negative staining.

Results: Positive staining was seen in 41 of 58 (70.7%) breast carcinomas with mammaglobin while only 24 (41.4%) cases showed immunoreactivity with GCDFP-15. In majority (over 90%) of the cases, the staining intensity and the number of cells staining were higher with mammaglobin compared to GCDFP-15. All tissue cores (tumor and normal) in the lung, stomach, colon, kidney and bladder arrays were negative. All cervical squamous cell carcinomas were also negative. Five of 80 (6.25%) melanomas, 1 of 40 (2.5%) ovarian papillary serous carcinoma and 23 of 59 (40%) endometrial carcinomas were also positive.

Conclusions: (1) Mammaglobin is a more sensitive marker than GCDFP-15 for breast carcinoma with comparable specificity to GCDFP-15 depending on the differential diagnosis and immunostaining patterns. (2) When the differential diagnosis for a metastatic carcinoma includes a breast primary, then a combination of mammaglobin and GCDFP-15 would be superior to GCDFP-15 alone. (3) The strong immunoreactivity for mammaglobin in a significant percentage of endometrial carcinoma requires further investigation, as it may be diagnostically useful in the female genital tract. (4) A small percentage of melanomas may stain with mammaglobin and therefore it is important to remember this diagnostic pitfall.

89 Pathologic Changes Associated with Electronic Brachytherapy

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Background: Electronic brachytherapy is an exciting new technology that utilizes a miniaturized x-ray source to apply radiation directly to a tumor bed without radioactive isotopes or heavy shielding. Accelerated partial breast irradiation can mimic the penetration and dose rate of 192Ir allowing delivery of a therapeutic dose directly to a tumor bed. The pathologic changes associated with traditional radiation therapy include changes in the terminal duct lobular units, changes in stromal fibroblasts and vascular changes. This study was performed to assess the pathologic changes seen following electronic brachytherapy in 4 test animals.

Design: Bilateral lumpectomies were performed on four healthy adult Nubian milk goats and balloon applicators were percutaneously inserted. Electronic brachytherapy was performed on one side only and twice daily radiation was given for five days starting on day 4. Each balloon in the contralateral breast cavity served as a control. Goats were euthanized 14 days after the final dose fraction and the breast tissue surrounding all cavities was resected, processed and analyzed. Selected sections were double immunostained with antibodies directed against MIB-1 and Caspase 3 (Biocare) to assess relative levels of proliferation and apoptosis.

Results: The pathologic changes seen in the breast tissue subjected to accelerated partial breast irradiation were similar to those described for traditional radiation therapy. Relative to the control breast, the treated breast revealed slight epidermal thickening, TDLU changes including epithelial atypia, inflammation, squamous metaplasia, fibrosis, atrophy, increased apoptosis and significantly increased proliferation in intralobular stromal tissue, ductal changes including intraductal hyperplasia and focal atypia and vascular changes including thickening and increased tortuosity of vessels.

Conclusions: Electronic brachytherapy is a minimally invasive procedure capable of delivering a therapeutic dose of radiation without the need for radioactive isotopes or a shielded environment. The procedure offers the ability to significantly reduce treatment time to 5 days and minimizes radiation expose to healthy tissue. The pathologic changes induced in the tissue surrounding lumpectomy cavities of Nubian milk goats are similar to those described for whole breast external beam radiation therapy.

90 Comparison of Breast Prognostic Markers When Obtained with the Cassi Biopsy Device Versus a 14 G Needle Core Biopsy

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Background: The Cassi is a new biopsy device that offers several attractive features to radiologists and surgeons. It is a large core, hand held device that is disposable and fully automated but unlike other devices, requires no capital investment or significant set-up time. Procedurally, the Cassi is unique. Once it is introduced into the lesion, using imaging guidance, the biopsy needle is rapidly frozen which immobilizes and secures the tissue to the biopsy needle. A cutting cannula rotates quickly over the needle to obtain the tissue sample. The purpose of this study was to assess if the cryo-assisted procedure in any way alters the immunohistochemical assessment of estrogen or progesterone receptor or the assessment of the HER2 gene by FISH.

Design: Twenty-six breast lesions were evaluated from 18 patients. Each lesion was biopsied with both the Cassi device and a standard 14 G needle. Device order was randomized such that half of the patients were first biopsied with the Cassi and half were first biopsied with the 14 G needle. All specimens were fixed in 10% neutral buffered formalin (Z-Fix), routinely processed and paraffin embedded. Estrogen and progesterone receptor were assessed immunohistochemically using the FDA cleared PharmDx kit (DakoCytomation). The status of the HER2 gene was assessed by FISH using the PathVysion assay (Abott Labs). All assays were scored in accordance with the package inserts.

Results: Eleven cancers were identified in ten patients. Ten of the lesions were not amplified by FISH and the HER2:CEP17 ratio was identical in 6 of the lesions and within 0.1 in the other four lesions. The one amplified tumor showed a ratio of 10.0 with the Cassi biopsy and 9.6 with the 14 G needle. Nine lesions showed identical Allred scores for ER, one showed a slightly lower Allred score on the Cassi biopsy and one showed a slightly higher Allred score. Six lesions showed an identical Allred score for PR, three showed a slightly lower score on the Cassi biopsy and two showed a slightly higher score. There were no discrepancies between positive and negative results.

Conclusions: The Cassi is an FDA cleared large core biopsy device that offers many advantages to surgeons and radiologists. The rapid freezing as part of the biopsy procedure does not alter the assessment of estrogen or progesterone receptor when performed with the Dako PharmDx kit for ER and PR, nor does it affect the assessment of the HER2 gene status when performed with the PathVysion assay.

91 Intact Image Guided Breast Biopsy Reduces Need for Subsequent Open Excision in Benign Proliferative Lesions

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Background: The diagnosis of some proliferative breast lesions, including atypical ductal hyperplasia (ADH), papilloma, and radial scar on a needle core biopsy usually prompts an surgical excision because of the possiblity of sampling error. In such cases, a lesion more significant than ADH is found on open biopsy in up to 20% of cases, depending on the size of the needle. The en bloc® biopsy device uses standard stereotactic guidance, and produces an intact spherical specimen. If the lesion is centrally placed within the specimen, and there is no mammographic evidence of the lesion post-biopsy, we hypothesize that an open biopsy will not reveal a more concerning lesion, and is unnecessary.

Design: Patients from three different institution underwent biopsy using the en bloc® system. Pathology reports were reviewed, and slides from cases that were diagnosed as proliferative lesions or low grade ductal carcinoma in situ were reviewed centrally. In addition to confirming the original diagnosis, the central pathology review evaluated the cases for thermal artifact, location of the lesion within the specimen (central versus peripheral), and whether determinant calcifications were present within the lesion. The central pathology review was blinded to the results of the subsequent surgical excision. Reports from subsequent surgical excisions were reviewed.

Results: From the three institutions a total of 32 benign lesions were biopsied using the en bloc® system. Diagnoses included proliferative lesions without atypia (n=20) atypical ductal hyperplasia (n= 7), atypical lobular hyperplasia (n=2), radial scar (n=1), and enlarged lobular units with columnar alteration (n=2) . Follow-up information regarding the subsequent surgical was available for 12 cases of proliferative lesions without atypia and 8 cases of atypical hyperplasia. When the lesion was centrally placed within the specimen, there were no upgrades at the time of open biopsy. Two examples showed atypical ductal hyperplasia at the edge of the en bloc® biopsy specimen. Open biopsy showed ductal carcinoma in situ.

Conclusions: Careful case definition with well-characterized lesions may obviate the need for surgical excision..

92 Expression of Potassium Channels (KC) in Normal, Hyperplasic and Carcinomatous Breast Human Tissue

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Background: Cell proliferation and apoptosis are responsible for maintaining the normal tissues homeostasis. Tumour cells possess various types of KC. They play important roles in the regulation of tumour cell proliferation and apoptosis.

Design: Immunohistochemistry was used to study the expression of six types of KC in hyperplastic (six), carcinomatous (ten , with or without positive lymph nodes) and normal (six) breast tissue. Selected KC antibodies included GIRK1, hEAG, BKCa, TASK2, Kv1.1 and Kv1.3 (Alomone, Haifa, Israel). Bcl2 antibody expression (clone124, Dakocytomation, USA) was evaluated in carcinoma cases. Electrophysiological data were obtained using the patch-clamp method on whole-cell system.

Results: The six types of KC were present in the epithelial compartment with an over expression for GIRK1 and hEAG in both cancerous and hyperplasic tissues as compared with the normal tissue. The expression of BKCa was marked in the normal and hyperplasic tissues while it decreased in cancerous tissue. Expression of both Kv1.1 and Kv1.3 was decreased in breast carcinomas in comparison with normal tissue. No immunostaining of TASK2 was observed in normal human breast , while the hyperplasic and cancerous samples showed respectively a high and a low staining. Moreover, we compared the presence and the absence of KC expression with lymph node metastasis. The expression of GIRK1 was similar while the expression of hEAG and BKCa was decreased in the lymph node metastatic tissues and the expression of Kv1.1 and Kv1.3 disappeared completely. The expression of the antiapoptotic protein Bcl2 was increased in all breast carcinomas. By a parallel electrophysiological study on primary culture cells functional KC were observed in breast carcinomas.
Conclusions: To our knowledge this is the first study of immunohistochemical expression of potassium channels in human breast tissue. GIRK1 and hEAG KC are strongly expressed in cancerous breast tissue and could possibly be used as pharmaceutical target. The downregulation of Kv1.1 and Kv1.3 in cancerous tissue indicates they might be useful as a biomarker for diagnosis of carcinomatous changes.

93 Evaulation of Phospho-mTOR (Mamalian Target of Rapamycin) Expression in Benign, Proliferative and Malignant Epithelial Lesions of the Breast

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Background: Mammalian Target of Rapamycin (mTOR) is a serine-threonine kinase of the cellular phosphatidylinositol 3-kinase (PI3K) pathway. This pathway is activated by various receptor tyrosine kinases as well as estrogen receptors. The PI3K/pAkt pathway is known to inhibit apotosis and to promote cell survival. mTOR is principle downstream target of PI3K/pAkt , and is associated with tumorigenic properties. mTOR mediates some of its actions through the downstream effector p70S6K. Rapamycin is a specific mTOR antagonist resulting in cell cycle arrest in G1 phase. In early clinical trials Rapamycin as well as its analogs have demonstrated impressive growth inhibitory effects. It is hypothesized that the phospho-mTOR (the phosphorylated activated form of mTOR) is increased in breast carcinoma compared to benign epithelial changes.
Design: Formalin-fixed, paraffin-embedded tissue from 253 cases were retrieve from the department files at Baystate Medical Center. Ten cases were benign lesions including adenosis, ductal hyperplasia without atypia, apocrine metaplasia; 31 cases ductal carcinoma insitu (DCIS), 208 infiltrating ductal carcininoma (IDC), 3 infiltrating lobular carcinoma (ILC) and 1 medullary carcinoma (MC). Tissue microarrays were created using a Beecher manual tissue arrayer. Five 0.8 cm cores were taken from each lesion. The expression of phospho-mTOR and its downstream regulator p70S6K (Cell SignalingTechnology) was studied by immunohistochemistry on an automated DAKO platform.
Results: Phospho-mTOR expression is present in 15.4% (32/208) cases of IDC, 48.4% (15/31) cases of DCIS, 100% (1/1) cases of MC, 0% cases of ILC (0/3) and 0% (0/10) in benign lesions of the breast . 8/48 (16.6%) of positive phospho-mTOR cases were also positive for p70S6K.
Conclusions: Phospho-mTOR is not expressed in benign lesions of the breast. However, phospho-mTOR is present in a subset of (15.4%) cases of IDC and almost in half (48.4%) of DCIS cases. This high percentage of DCIS cases associated with expression of phospho-mTOR suggests that phospho-mTOR may be involved in the development, maintenance or progression of DCIS. mTOR expression is associated with response to Rapamycin and its analogues by inhibiting cell cycle proliferation. These findings suggest that further evaluation of phospho-mTOR expression and associated regulatory proteins may provide new treatment strategies for DCIS.

94 MRI Directed Breast Excisions for Lesions Not Visualized by Mammography or Ultrasound – Frequency of Grossly Occult Malignant Diagnoses

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Background: Magnetic resonance imaging (MRI) is increasingly being used to evaluate patients for breast lesions, both at large academic centers and in the community. Pathologists are encountering breast excisions for lesions identified through MRI that cannot be detected by ultrasound, mammography, or physical examination. Significantly, suspicious lesions are identified on MRI by their contrast enhancement characteristics; a technique that cannot be performed on a surgically removed specimen. This study focuses on the gross and radiographic evaluation of these specimens, and makes data driven recommendations for proper evaluation.
Design: 44 MRI directed wire localized excisions performed on 42 patients were examined retrospectively, with special attention paid to radiographic, gross, and microscopic findings.
Results: Malignant diagnoses were rendered in 14/44 specimens (32%). These included invasive carcinoma (9/44 specimens, mean size: 0.5 cm), which was identified grossly in only 2/9 cases and by specimen radiograph in only 1/9 case. Four cases of ductal carcinoma in situ were identified, two of which had microinvasion. Lymphoma was diagnosed in one case. In the 10/44 specimens with a grossly identified lesion, the majority (8/10) were benign (4 fibroadenomas, 2 were radial sclerosing lesions, 1 papilloma, and 1 scar), while only 2 were malignant (2 invasive carcinomas, mentioned previously).
Conclusions: MRI directed excisions are extremely challenging specimens for the practicing pathologist. In this series, >80% of patients (35/42) were at high risk (i.e. a family or personal history of breast carcinoma), and it is not, therefore, surprising that there would be a relatively high proportion of malignant diagnoses (32%). Grossly identified lesions, in the few cases where one is present, do not typically correlate with malignancy, but are rather more frequently associated with benign diagnoses. In that neither gross specimen evaluation nor specimen imaging with radiography can reliably identify these MR-localized lesions, it is recommended that specimens be entirely submitted for histologic examination.

95 Gene Expression Profiling of NMU-Induced Rat Mammary Tumors: A Model of Human Breast Cancer Progression

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Background: The ability of microarray analysis to elucidate the genes responsible for breast cancer progression has been hindered by both the genetic complexity of the disease and, the inability to follow the progression of a single in situ cancer. Using a carcinogen-induced animal model of mammary tumorigenesis combined with a cross species microarray analysis, we have previously shown that these rat mammary tumors share expression profiles with ER positive, low-grade human breast cancers. We now have further expanded this model to identify genes that are unique to the acquisition of an invasive phenotype.
Design: Female Wistar-Furth rats were given a single intraperitoneal injection of N-methyl-N-nitrosourea. The resulting tumors (n=11) were serially transplanted into syngeneic rats through five generations (n=65). All tumors were scored according to the degree of invasion, as defined by histology, loss of basement membrane and alterations in P63 staining. Lungs, liver, kidneys, and spleen were examined histologically for metastases. The epithelial differentiation of malignant, invasive cells was confirmed in a subset of tumors using vimentin and pan-cytokeratin staining. Gene expression profiles of bulk tumor samples were generated using rat Affymetrix arrays containing 15866 genes.
Results: All serially transplanted tumors progressed from a ductal in situ neoplasm to an invasive tumor, some with distant metastases (n=7) and local tissue invasion (n=18). Unsupervised hierarchical clustering identified two major clusters, highly invasive tumors and tumors with low invasive scores. A unique transplant series was identified with a basal-like phenotype, which clustered with highly invasive tumors. Supervised clustering yielded several genes involved in cell adhesion and invasion. A logarithmic linear regression analysis identified genes that were unique to invasion independent of the transplant lineage.
Conclusions: Using an animal model of invasive breast cancer and gene expression profiling, a molecular signature of invasion is described that may have prognostic and therapeutic implications in human breast cancer. This study has overcome a difficult limitation of human microarray analyses by following the genetic changes acquired during progression of a single tumor.

96 Epidermal Growth Factor Receptor (EGFR) Protein Overexpression Is Associated with Gene Amplification in Metaplastic Carcinomas of the Breast (MCB)

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Background: Metaplastic carcinomas of the breast (MCB) represent a morphologically distinct subgroup of tumors, which are often negative for ER, PR and Her2/neu, limiting treatment options for patients with advanced disease. Prior studies have reported EGFR protein expression in some MCB, suggesting a new potential therapeutic target. We sought to determine the frequency of EGFR protein overexpression in MCB and to correlate it with gene amplification.
Design: Tissue microarray blocks were constructed from 32 MCB (11 tumors of purely spindle cell morphology (MCB-SC) and 21 mixed tumors (MCB-MIX) composed of invasive ductal carcinoma with squamous, spindle cell or heterologous components). The tumors were assessed for immunoreactivity for cytokeratins (AE1/AE3, 34BE12, Cam5.2, CK 5/6), ER, PR, Her2/neu (Herceptest, DAKO), and EGFR (DAKO). Her2/neu and EGFR immunopositivity was scored as 0 to 3+, according to the manufacturer's recommendations. EGFR gene amplification was evaluated by chromogenic in situ hybridization (CISH) using commercially available kit (Zymed). Tumors were interpreted as positive for gene amplification when the average number of gene copies was > 5 per nucleus.
Results: All tumors with pure spindle cell morphology were immunoreactive for at least one cykeratin antibody. All MCB were negative for ER, and only 2 tumors showed positivity for PR and Her2/neu. The results of immunohistochemistry and CISH for EGFR are shown below.

Tumor Type	EGFR (0 to 1+)	EGFR (2+)	EGFR (3+)	EGFR-amplified (CISH)
MCB-SC	5/11 (45%)	6/11 (55%)	0%	1/11 (9%)
MCB-MIX	16/21 (76%)	2/21 (10%)	3/21 (14%)	3/21 (14%)
Total MCB	21/32 (66%)	8/32 (25%)	3/32 (9%)	4/32 (12.5%)

EGFR gene amplification correlated with the immunohistochemistry results and was detected in all 3+ positive tumors (all of them MCB-MIX) and in one 2+ MCB-SC.
Conclusions: In this study, approximately one third of MCB were positive for EGFR by immunohistochemistry, and a subset of tumors showed both EGFR overexpression and gene amplification. These results suggest that some metaplastic carcinomas may be candidates for treatment with EGFR inhibitors.

97 Estrogen Receptor Negative Breast Cancer: Biology and Phenotypic Characteristics

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Background: Estrogen receptor (ER) has recently been shown to have two isoforms, ER-alpha and beta, with ER-beta being distinctly different from ER-alpha. Currently, ER-alpha is the main diagnostic and therapeutic receptor. ER-alpha negative tumors are considered aggressive and do not benefit from antiestrogen treatment. Our preliminary study showed ER-beta expression in ER-alpha negative tumors, suggesting a possible crucial role. This study aims to investigate the expression of ER-beta and

other biomarkers, and morphology in ER-alpha negative tumors to determine unique phenotypic features and associations.
Design: Tissue microarray (TMA) was prepared from paraffin embedded blocks of 209 infiltrating breast cancers. Immunohistochemistry (IHC) was conducted on TMA slides, using a Dako immunostainer for ER-alpha (ID5, Dako), ER-beta (ERb88, 1:40, Biogenex, CA), PR, p53, Ki-67, Her-2/neu, E-Cadherin and Cathepsin D. ER-alpha negative tumor was defined as a complete absence of nuclear staining in all cancer cells, and positive as > 5% nuclear and/or cytoplasmic staining of ER-alpha and other biomarkers. A statistical significance was determined by Chi-square test.
Results: Eighty four (40.2%) cases were ER-alpha negative tumors. The majority were ductal type (88.8%) followed medullary carcinoma (5.6%) and other types (5.6%, apocrine/duct/lobular) and most were of a high nuclear grade (66.9%, grade 3; 28.4%, grade 2; 4.7%, grade 1). Tumor necrosis and angiolymphatic invasion was present in 32% and 22.6%, respectively. ER-alpha negative tumors showed a significantly higher expression of Ki-67 (32 vs 17%, p<0.01), Her-2/neu(27 vs 17%, p< 0.001), Cathepsin D(62 vs 44%, p<0.025) and lower expression of PR (14 vs 71%, p< 0.001) than did ER-alpha positive tumors.Expression of E-Cadherin was lost or aberrant in 16 % of cases. ER-beta was singly expressed in 46% (38/84) of the ER-alpha negative tumors and was associated with the expression of p53 and Ki-67.
Conclusions: ER-alpha negative tumors appear to have distinct morphologic and biomarker characteristics from ER-alpha positive tumors. They are associated with high nuclear grade, PR negativity and poor prognostic biomarkers, Her-2/neu, Ki-67, p53 and Cathepsin D. The marked presence of ER-beta expression in ER-alpha negative tumors suggests that ER-beta may be a key determinant of estrogen signaling pathways, and behavior patterns in these tumors. The diagnosis and therapy of breast cnacer may benefit from greater focus on the role of ER-beta isoform, particularly in ER-beta positive / ER-alpha negative tumors.

98 Interobserverer Variability of Lobular Neoplasia

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Background: Lobular neoplasia (LN) and infiltrating lobular carcinoma (ILC) are presumed to have distinct biological and phenotypical tumor characteristics, and clinical implications different from those of ductal neoplasia (DN) and infiltrating duct carcinoma (IDC). The histological diagnoses of either DN / IDC or LN / ILC can be problematic in tumors with low grade morphological features. Interpretations can vary among pathologists. E-Cadherin has been shown to be useful in distinguishing LN / ILC on the basis of complete membrane staining. In this study, the accuracy and variability of histomorphological interpretations of LN / ILC on routine H&E were compared to E-Cadherin staining as a gold standard.
Design: One hundred sixty-five cases of breast lesions previously diagnosed as LN / ILC during the years 1998 to 2002 were randomly selected from the departmental files. Three surgical pathologists reviewed the slides in a blinded manner for histopathological and E-Cadherin staining interpretation. E-Cadherin staining was conducted on the paraffin-embedded sections of the breast lesions using two different E-Cadherin antibodies (ECH, prediluted, Cell Marque, AK and NCH-38, 1:25 dil, Dako, CA) and DAKO automated immunostainer after antigen retrieval. Appropriate controls were included. Only complete E-Cadherin membrane reactivity of epithelial cells was recorded as positive and complete absence as negative.
Results: The histological diagnoses of LN / ILC were confirmed by E-Cadherin stain in 88.9 % (145/163): 73% (107 /145) of the LN / ILC were diagnosed using routine H&E stain by three pathologists, and 20.7% by two pathologists and 6.3% by one pathologist. E-Cadherin stain changed the diagnoses of LN or ILC to DN or IDC in 11.2% (18 /163). E-Cadherin membrane immunoreactivity was at times heterogenous and did not always show complete membrane staining. When 73 cases of LN / ILC were stained with antibodies from two different manufactureres, there was a 5.6 % discrepancy.
Conclusions: The study confirms variability in interpreting LN / ILC using routine H&E stain among pathologists, and proves that E-Cadherin staining is useful in classifying LN / ILC versus DN / IDC. The standardization of E-Cadherin antibodies and the interpretation of E-Cadherin staining is needed, and will become essential to the management of LN / ILC.

99 CA IX Is an Independent Predictor of Poor Prognosis in Breast Carcinoma: Results from a Population Based Study of 4150 Breast Cancers

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Background: Carbonic Anhydrase 9 (CAIX) is a membrane-associated carbonic anhydrase which is strongly induced by hypoxia. Increased expression of CAIX has been shown in a wide spectrum of tumor types and clinical studies have shown a clear relationship between CAIX levels in tumors and poor prognosis.
Design: A population-based tissue microarray was constructed of 4150 cases of invasive breast carcinoma. These tumors were newly diagnosed and referred to British Columbia Cancer Agency during 1986-1992. Both stromal cell and tumor cell expression of CAIX was assessed and scored as either positive or negative. The staining was then correlated with prognostic parameters including patient age, tumor size, grade and nodal status. Disease specific survival and relapse free survival analyses were done, and log-rank test was used to estimate the significance between survival estimates. Cox proportional hazard model was used for multiple regression.
Results: There was significant correlation between CAIX positive staining and larger tumor size, higher tumor grade and younger age of patient. There was no correlation between CAIX positive staining and nodal status. CAIX positive staining was associated with worse disease free survival (4.12E-16) and disease specific survival (p=9.6E-18). In Cox regression model CAIX (HR 1.47 CI 95% 1.26-1.71, p=1.21E-6) is independent of age, tumor size, grade, nodal status and immunohistochemical expression of HER2 and ER.CAIX showed significant inverse correlation with ER status (Kendall's tau-b=-0.28, p=2.23E-47).
Conclusions: In our population-based series we have demonstrated that CAIX is a significant worse prognostic marker of relapse free and disease specific survival in patients with invasive breast cancer. Expression of CAIX also shows significant association with larger tumor size, higher grade and younger patient age but not with nodal status.

100 The Effect of a Positive Family History on Breast Cancer Risk in Women with Prior Benign Breast Disease

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Background: Prior clinical and epidemiologic studies have demonstrated an association between histologic category of benign breast disease (BBD) and risk of subsequent breast cancer. However, the influence of a positive family history (FH) of breast cancer on breast cancer risk among women with biopsy-confirmed BBD is less certain.
Design: To address this, we conducted a case-control study of BBD and breast cancer risk nested within the Nurses Health Study. Cases were women with breast cancer who had a previous benign breast biopsy (n=395). Controls were women who also had a previous biopsy-confirmed diagnosis of BBD, but who were free from breast cancer at the time the corresponding case was diagnosed (n=1621). Controls were matched to cases on year of birth and year of benign breast biopsy. Histologic sections of the benign breast biopsies were reviewed and categorized as showing either non-proliferative lesions, proliferative lesions without atypia, or atypical hyperplasia (AH).
Results: Among women with BBD, those with a FH of breast cancer in a first-degree relative (mother or sister) had an increased breast cancer risk when compared to women without a FH (Adjusted Odds Ratio [O.R.] 1.5; 95% CI, 1.2-2.0). When compared with women with non-proliferative lesions and no FH, women with proliferative lesions without atypia and a positive FH had a slightly higher breast cancer risk (O.R. 2.4; 95% CI, 1.6-3.6) than those without a FH (O.R. 1.5; 95% CI, 1.1-2.0), and this difference approached statistical significance (p=0.07). In contrast, a FH of breast cancer did not significantly increase the risk among women with AH. The O.R. for the development of breast cancer was 4.2 (95% CI, 2.8-6.2) for women with AH and no family history and 5.4 (95% CI, 3.0-9.6) for those with AH and a positive FH (p=0.47).
Conclusions: In this study, a family history of breast cancer in a first-degree relative slightly increased the breast cancer risk among women with proliferative lesions without atypia, but there was no substantial additive effect of a positive family history on breast cancer risk among those with atypical hyperplasia.

101 Magnitude and Laterality of Breast Cancer Risk in Women with Atypical Hyperplasia of Ductal and Lobular Types

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Background: Women who have had a benign breast biopsy showing atypical hyperplasia (AH) are at increased risk for the development of breast cancer. However, the details of the relationship between histologic type of AH (i.e., atypical ductal hyperplasia [ADH] vs atypical lobular hyperplasia [ALH]) and the magnitude and laterality of breast cancer risk are less well-defined.
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 invasive breast cancer who had a prior benign breast biopsy (n=316). Controls were women who also had a prior benign breast biopsy but who were free from breast cancer at the time the corresponding case was diagnosed (n=1621). Benign breast biopsy slides were reviewed by pathologists blinded to case/control status and were categorized as showing either non-proliferative lesions, proliferative lesions without atypia, or AH. Those showing AH were further categorized as ADH or ALH using the criteria of Page et al.
Results: Compared with women with non-proliferative lesions, the adjusted odds ratio (OR) for invasive breast cancer among all women with AH was 3.9 (95% CI, 2.7-5.8). However, the magnitude of the breast cancer risk differed according to histologic type of AH. The OR for the subsequent development of breast cancer among women with ALH (28 cases; 43 controls) was 5.2 (95% CI, 3.0-9.1) whereas the OR for those with ADH (33 cases; 104 controls) was 2.8 (95% CI, 1.7-4.4); this difference approached statistical significance (p=0.08). Overall, 58.9% of the breast cancers that developed in women with AH were in the ipsilateral breast. While the risk of ipsilateral breast cancer was higher among women with ALH than those with ADH (62.5% vs 56.0%), this difference was not statistically significant (p=0.64).
Conclusions: Women with AH in a benign breast biopsy are at a substantially increased risk for the development of breast cancer, but the risk appears to be greater among those with ALH than among those with ADH. Given that only about half of the subsequent cancers that develop in women with AH occur in the ipsilateral breast, these lesions are best viewed as markers of a generalized increase in breast cancer risk for the purposes of clinical management.

102 The Spectrum of Molecular Morphologic Abnormalities of the E-Cadherin-Catenin Complex in Pleomorphic Lobular Carcinoma of the Breast

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Background: Pleomorphic lobular neoplasia (PLN) encompass the high grade variants of in situ (PLCIS) and invasive lobular carcinoma (PLC). E-cadherin, an invasion suppressor gene, codes for a transmembrane protein that functions in intercellular

adhesion. The E-cad internal domain binds with alpha, beta, gamma and p120 catenins to anchor the E-cad-catenin complex (ECCC) to the actin cytoskeleton of the cell. The E-cad gene is mutated in PLN, resulting in loss of the extracellular domain and lack of membrane staining. This study focuses on the molecular morphologic spectrum of the ECCC in PLN and compares it to the ductal phenotype.

Design: Thirteen cases of PLN were studied. All cases had PLCIS; 5 of these had PLC and two had invasive duct carcinoma (IDC). Slides were reviewed, and IHC with antibodies to e-cad, and catenins alpha, beta, gamma and p120 was performed on the Ventana Benchmark XT. Patterns of immunostaining were recorded and compared to normal breast epithelium and to IDC.

Results: Normal breast epithelium and IDCs had intense linear cell membrane immunostaining with all five antibodies. PLN patterns of membrane immunostaining (MIS) of antibodies included negative(N), beaded (B), or dot-like(D). IS patterns were identical for PLCIS and PLC. Cytoplasmic immunostaining (CIS) patterns included finely granular (FG), coarse paranuclear granules(CPG) or diffuse intense cytoplasmic staining (DC). A proportion of cells with no MIS or CIS were seen in each case, but the dominate IS patterns were as follows:

No. Cases 13	ECAD	α cat	β cat	γ cat	p120 cat
Pattern: MIS	9N, 4B	1B, 12N	2B, 11N	6B, 7N	2D, 10N
Pattern: CIS	11FG, 2D	12FG, 1N	13CPG	13FG	13DC

Conclusions: (1) Compared to the ductal phenotype, PLN shows displacement of the ECCC from the cell membrane. (2) Dominant patterns of granular CIS suggests cytoplasmic relocation of the ECAD internal domain along with alpha, beta and gamma catenins . (3) Beaded catenin MIS parallels beaded ECAD MIS. (4) Characteristic morphologic IS patterns, with some variation, reflect a variety of ECCC molecular abberations in PLN that probably reflect a series of ECCC gene mutations.(5) Catenin IS abnormalities are not seen in the presence of ECAD membrane IS suggesting that an ECAD abnormality is the primary event that leads to a disrupted ECCC (6) Compared to the linear ductal phenotype of P120 MIS, the diffuse cytoplasmic P120 IS of PLN may be diagnostically useful to distinguish lobular from ductal lesions.

103 Results of a Long Term Follow-Up Study of 115 Patients with Flat Epithelial Atypia

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Background: There are still controversies about the management of patients with FEA/DIN 1A corresponding to columnar cell hyperplasia with atypia (clinging carcinoma of the monomorphic type) since there are few available clinical follow-up studies. The objective of our work was to analyze the absolute risk of developing a subsequent cancer in patients with such lesions.

Design: From 1980 to 1997, 115 patients underwent surgical biopsies (SB) for "clinging carcinoma of the monomorphic type" as a single lesion (median follow-up: 160 months). SB were entirely sectioned into 2mm thick slices (median number of blocks per SB: 26). The mean size of the lesion was 6mm and the mean extension (percentage of blocks with the lesion: 17%). Radiotherapy was performed in patients with an extensive lesion (n=45).

Results: There were 3 infiltrating ductal carcinomas/IDC in the same breast (1 in the group of patients with radiotherapy) 1, 7 and 12 years later, 3 FEA in the same breast at 4 and 5 years, and 1 ductal carcinoma in situ/DCIS in the contra-lateral breast at 10 years.

Conclusions: The two IDC occurring 7 and 12 years later were probably new cancers and the one occurring one year later might have been initially missed at the time of the diagnosis of FEA since it occurred before the use of needle localization. The absolute risk of developing invasive cancer after a diagnosis of FEA is very low. When FEA is isolated further treatment other than excision is not necessary.

104 Diagnostic and Prognostic Utility of Molecular Markers in Bilateral Synchronous Breast Carcinoma

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Background: Approximately 5-10% of all breast cancer (BC) are bilateral (predominantly metachronous) with only 1% occurring synchronously. Histologic criteria is limited in determining if the synchronous tumors represent two primaries or a metastasis to the contralateral breast when the BCs are of similar type. Patients with synchronous ipsilateral primary carcinomas have a more favorable prognosis than patients that have BC metastatic to the contralateral breast. In this study, we investigated if molecular analysis of bilateral synchronous BC can be an aid in separating two primary tumors versus a metastasis involving the contralateral breast.

Design: This study examined the genetic alterations at 15 polymorphic DNA markers in the DNA of tumors of 17 patients with breast carcinoma, including 12 patients with bilateral synchronous BC and control group of 5 cases with infiltrating ductal carcinoma and regional lymph node metastases. After review of each case, one slide representing the tumor from each breast or lymph node (total 34 slides) was selected and 4-8 target areas were microdissected and DNA was extracted. Mutations were quantitatively determined to detect loss of heterozygosity (LOH) and microsatellite size alterations (MA) for a broad panel of 15 markers (1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q) using polymerase chain reaction (PCR) with labeled followed by capillary electrophoresis. The tumors were classified as de novo or metastasis based on 3 levels of concordance: (1) marker affected - tumors were considered concordant if 50% or more of the same markers were mutated, (2) same gene copy affected, and (3) temporal sequence of mutation.

Results: In 5 patients with metastases to the regional lymph node, the primary BC and metastasis shared the same mutations. In 12 patients with bilateral synchronous BC, mutations were discordant, supporting the diagnosis of synchronous de-novo bilateral primary BCs. H and E failed to provide accurate diagnosis (dx) in 7 of 10 bilateral BCs based on histological type and grading.

Conclusions: Our study showed that synchronous bilateral BCs represent two separate primaries. The application of molecular analysis technique plays an important role in the differential dx of synchronous primary carcinomas verses a BC with metastasis to the contralateral breast. Routine pathological examination can provide an accurate diagnosis only when different histologic BCs are present.

105 Risk of Carcinoma in Papillomas with Atypical Ductal Hyperplasia (ADH)

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Background: Papillomas of the breast with ductal neoplasia may be interpreted as papilloma with ADH (PADH) or papilloma with ductal carcinoma in situ (PDCIS) based on the perception of extent of the neoplastic proliferation. There is no currently defined standard terminology for these lesions because of limited outcome data. A diagnosis of PADH leads to excision alone, where as PDCIS may result in unnecessary radiation therapy or mastectomy.

Design: The slides of 47 papillomas with ductal neoplasia limited to the papilloma (45 solitary, 2 multiple) were reviewed and the size of the papilloma and percentage of neoplastic proliferations within it were assessed. In 36 cases we also analyzed the expression of high molecular weight cytokeratin CK5/6 to aid in assessing the proportion of ductal neoplasia. Calponin stain was done to delineate myoepithelial cells. We followed up 43 cases diagnosed as PADH and 4 cases diagnosed as PDCIS to ascertain outcome.

Results: The follow-up interval ranged from 6 mo to 9 y (mean 4.5 y). One of 43 (2.3%) patients with PADH developed well differentiated invasive ductal carcinoma (IDC) in the same breast 2 years later in a different location (size of the papilloma was 3 mm and contained 30% neoplastic proliferation). The other 42 patients had negative follow up for malignancy in both breasts. Sizes of papillomas varied from 0.2 – 3 cm and the neoplastic proliferations varied from 5 – 95%. Two patients had past history of IDC in the same breast (10 and 8 years earlier); one had concurrent contralateral IDC. All 4 patients with PDCIS had negative follow up. Sizes of papillomas varied from 0.2 – 1.3 cm and percentage of ductal neoplasia varied from 60 – 95%. One patient had previous IDC (1 years earlier). Neoplastic proliferations were uniformly negative for CK5/6 in all 36 cases and their quantity appeared to be greater than that visualized in H&E sections (5-30% more in 20 cases). CK5/6 and calponin also high-lighted the background myopeithelial cells.

Conclusions: 1. There is no clear distinction between ADH and DCIS when they occur in a papilloma. 2. Size of papilloma or the quantity of neoplastic proliferations within it do not seem to have a prognostic significance. 3. Although PADH/PDCIS can be associated with, precede, and/or follow invasive ductal carcinoma, the risk of subsequent invasive carcinoma incidence is low in this series (1.9% combined). 4. To avoid over treatment of these relatively low risk lesions, a conservative diagnosis of 'papilloma with ADH' is probably more appropriate than 'DCIS', when the neoplastic proliferation is limited to the papilloma.

106 Frequency of Clinically Occult Intraepithelial and Invasive Neoplasia in Reduction Mammoplasty Specimen. A Study of 517 Cases

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Background: The frequency of clinically occult intraepithelial and invasive mammary neoplasias is not well known among women less than fifty years of age. Reduction mammoplasty is an increasingly frequent procedure performed not only for the treatment of macromastia, but also for achievement of symmetry in patients with breast carcinoma. A large number of such specimen was reviewed to gain some insight into the development of mammary neoplasia.

Design: Slides from a total of 546 consecutive reduction mammoplasties performed over a 15-year period (June 1990-June 2005) were reviewed. Females of all ages were included regardless of concurrent or previous history of breast cancer, but the data for the 21 women with cancer and unilateral mammoplasty who had surgery for achievement of symmetry were analyzed separately. Eight cases with a diagnosis of gynecomastia were excluded.

Results: The mean age of the 517 women with bilateral reduction mammoplasty was 35 years (range 15-80 years); the mean weight of the samples was 817 grams (range 84-4540 grams; 17 cases had no weight recorded). A total of 517 cases were subclassified into 3 age groups: less than 40 years (352 cases), 40-50 years (108 cases) and over 50 years (57 cases). Among all cases, 69 (13.3%) low risk DIN/IDH, 4 (0.7%) hemangiomas and 1 (0.2%) tubular adenoma were identified. In addition, 10 cases (1.9%) of DIN 1(3 Flat type, 4 AIDH, and 3 combined), 1 (0.2%) DIN 2/DCIS, and 11 (2.1%) LIN (9 ALH and 2 LCIS) were identified. One (0.2%) clinically occult tubular carcinoma was discovered, in a patient over 50 years old. The most common pathologic finding was low risk DIN (IDH) 69/517 (13.3%). Interestingly, the percentages of low risk DINs and DIN 1, were found to increase with age from 7.4 % to 31.58% and 1.4% to 7%, respectively. In contrast, the frequency of LIN was inversely proportional to age: 2.3% (less than 40 years of age), 1.9% (40-50 years) and 1.8% (over 50 years).

Conclusions: These data confirm the low frequency of clinically occult intraepithelial and invasive neoplasia identified in breast reduction mammoplasty specimens, the majority occuring in women over 50 years of age. Therefore, it is reasonable to suggest that specimens from a higher risk group (patients older than 50 years and/or previous history of breast carcinoma), be inked for orientation and margin assessment.

107 Sentinel Lymph Node Biopsy Following a Diagnosis of Ductal Carcinoma In Situ on Needle Core Biopsy

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Background: Sentinel lymph node biopsy (SNB) is an acceptable alternative to axillary dissection for the staging of breast cancer. The procedure has shown low false negative rates and decreased morbidity compared with axillary dissection. The use of SNB in the setting of ductal carcinoma in situ (DCIS) is controversial, particularly in the setting of non-operative diagnosis. This is due to underestimation of invasive disease on needle core biopsy (NCB) and doubt as to the accuracy of SNB if primary surgery to the breast has already been performed. In 2003 our institution adopted a policy of performing SNB at the time of definitive surgery for all cases of DCIS diagnosed by NCB. We present our experience of 60 cases.

Design: We retrospectively analysed our database of symptomatic and screen detected cases of DCIS, diagnosed by NCB, for the period January 2003 to July 2005. Pathology reports of NCB and all subsequent surgical procedures were analysed to determine grade of DCIS on NCB, presence of invasion/microinvasion on subsequent resection and status of the sentinel lymph node. The sentinel lymph nodes were examined at multiple levels with haematoxylin and eosin and immunohistochemical staining for cytokeratin (CAM 5.2) for micrometastases, macrometastases and isolated tumour cells (ITC). Lymph nodes with ITC were classified as negative in accordance with the TNM classification of malignant tumours (6^{th} edition).

Results: 60 patients received a diagnosis of DCIS on NCB during the study (44 screen detected, 26 symptomatic). 22 patients (37%) had invasive disease (ductal or lobular carcinoma) on resection. NCB results for these patients showed 11 high grade, 11 intermediate grade and no low grade DCIS. A further 3 patients (5%) had microinvasive disease. All patients had at least one sentinel lymph node biopsied. 4 patients had a positive SNB (2 micrometastases and 2 macrometastases), of whom, 3 had invasive ductal carcinoma on excision and only 1 had pure DCIS. A further 4 patients had ITC detected by immunohistochemistry.

Conclusions: Lymph node metastases are rare in pure DCIS (1/35 cases in our study). However, the high rate of invasive carcinoma present in resection specimens of patients with DCIS only on NCB (22/60) warrants the practice of performing SNB at the time of definitive surgery. The use of immunohistochemistry on sentinel lymph nodes increases detection of ITC, the significance of this is not as yet well defined.

108 Extensive Retraction Artifact Correlates with Lymphatic Invasion and Nodal Metastasis in Human Breast Carcinoma

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Background: Retraction artifact resulting in clear spaces around tumor cell nests are frequently seen in formalin-fixed paraffin-embedded histologic material and may present difficulty in distinguishing such foci from lymphovascular invasion. We noticed that such retraction artifact is much more common around groups of breast cancer cells compared to benign acini, and when extensively present, metastasis to axillary lymph nodes is often seen. We decided to perform a systematic study to test our hypothesis that the presence of extensive retraction artifact around tumor cells correlates with lymphatic invasion and nodal metastasis.

Design: Two hundred and twenty-eight cases of stage pT1 and pT2 invasive breast carcinomas were selected for the study. All H&E stained sections were reviewed to confirm tumor type and grade. The presence and extent of retraction artifact around tumor cell nests and the presence of lymphatic invasion was determined. Lymphatic invasion and micropapillary features in carcinomas were confirmed by D2-40 and epithelial membrane antigen immunostaining, respectively.

Results: Axillary node dissection was performed in all cases. The median number of nodes removed was 15 (range 5 - 45). Nodal metastasis was present in 50 cases; the median number of positive nodes was 2 (range 1 - 15). The correlation of the extent of retraction artifact with various pathologic tumor features are summarized in the table.

Conclusions: Our results suggest that prominent retraction artifact in breast carcinomas highly significantly correlates with lymphatic invasion and nodal metastasis. We propose that this phenomenon, currently simply considered an artifact of fixation, is actually the result of certain biologic changes in tumor cells, i.e. loss of adhesion mechanisms, which may contribute to tumor progression and spread.

Correlation of retraction artifact with pathologic tumor features

			Percent retraction artifact		
		N	Median	Mean ± SEM	p*
Tumor type	Ductal	188	5	18.3 ± 1.8	0.0041
	Lobular	17	0	6.8 ± 4.7	
	Mixed	23	0	5.9 ± 2.0	
Tumor grade	Low	62	0	2.5 ± 1.3	< 0.0001
	Intermediate	97	5	17.4 ± 2.4	
	High	68	20	27.2 ± 3.2	
pT stage	1A	21	0	6.0 ± 4.0	< 0.0001
	1B	65	0	6.6 ± 1.7	
	1C	98	10	20.5 ± 2.5	
	2	44	15	26.3 ± 4.3	
Lymphatic invasion	Absent	149	0	5.6 ± 1.2	< 0.0001
	Present	79	30	36.2 ± 2.8	
Nodal metastasis	Absent	178	0	11.2 ± 1.5	< 0.0001
	Present	50	27.5	34.0 ± 3.8	

* Kruskal-Wallis test

109 Immunohistochemical Distribution of Thyroid Hormone Receptor in Non-Neoplastic Breast Tissue and Ductal Carcinoma

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Background: The interrelationship between thyroid hormone (TH) and breast carcinoma remains uncertain. TH appears to have an estrogen-like effect on breast cancer cell lines, and extractable thyroid hormone receptor (THR) has been measured in human breast cancers. Recently, primary hypothyroidism was reported to be associated with a reduced incidence of breast carcinoma and more indolent disease. Given this clinical implication, the purpose of this study was to describe the distribution of THR isoforms in benign breast tissue and ductal carcinomas as detected by immunohistochemistry (IH).

Design: IH staining for two THR isoforms ($\alpha 1/\alpha 2$ and β) was performed on 11 reduction mammoplasties and 18 cases of infiltrating ductal carcinoma (IDC). Ten of the IDCs were known estrogen and progesterone receptor positive, and 8 were negative by IH. Four of the IDCs were known HercepTest 3+ positive, and the remainder were negative. Nine of the IDCs were Bloom-Richardson grade III, 8 were grade II, and 1 was grade I. THR IH staining results were recorded as positive or negative, and the percentage of positive cells was noted.

Results: In all 11 reduction mammoplasties, ductal epithelial cells and myoepithelial cells showed nuclear staining for both THR isoforms. The percentage of cells staining ranged from 80 to >90% for both cell types. The percentage of cells staining appeared consistent throughout any given tissue section. Staining within lobular epithelium did not differ from that within ductal epithelium. Apocrine ductal cells stained similarly to non-metaplastic epithelium. In addition to epithelial and myoepithelial cells, nuclear staining for both isoforms was observed in variable numbers of fibroblasts, smooth muscle cells, endothelial cells, adipocytes, and inflammatory cells. All 18 IDCs demonstrated positive nuclear staining for both THR isoforms, with the percentage of positive cells ranging from 60 to >90%. Ductal carcinoma in-situ (DCIS) was present in 13/18 cases of IDC. All cases of DCIS showed positive nuclear staining for both THR isoforms, with the percentage of positive cells also ranging from 60 to >90%. Breast parenchyma adjacent to IDC stained similarly to reduction mammoplasty tissue.

Conclusions: By IH staining, THRs appear to be substantially present within nuclei of benign breast tissue as well as ductal carcinomas, irrespective of grade, hormone receptor status, or Her2neu status. This study is the first to characterize the IH distribution of THR in benign breast tissue and ductal carcinoma.

110 D2-40 Expression in Cutaneous Angiosarcoma Arising after Radiotherapy Treatment of Breast Carcinoma

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Background: Stewart-Treves syndrome (cutaneous angiosarcomas) occurring as a complication of radiation therapy for breast cancer are uncommon. In the absence of specific positive marker for lymphatic or vascular endothelia the precise histogenesis of Stewart-Treves syndrome has not been possible. D2-40 is a novel monoclonal antibody that reacts with a fixation-resistant epitope in lymphatic endothelium. In this study, we investigated the expression of D2-40 in angiosarcoma occurring as a complication of radiation therapy for breast cancer, in comparison to other commonly used endothelial immunohistochemical (IHC) markers.

Design: Archived paraffin embedded tissue from 25 cases of angiosarcoma occurring as a complication of radiation therapy for breast cancer were retrieved from the hospital computer database in the period between 1995-2000. One tissue block from each case was submitted for the IHC markers CD31, CD34, CD105 (endoglin) and D2-40. Twelve cases of non-radiation associated-angiosarcoma arising from different sites and 10 cases of hemangioma were also included as control group. Immunostains were performed on an automated immunostainer with appropriate positive and negative controls. Cases were screened by two pathologists and scored as positive or negative.

Results: Angiosarcomas arising in the breast were positive in 21/25 (84%) for D2-40, 9/25 (36%) for endoglin, 12/25 (48%) for CD34, 11/25 (44%) for CD31. Staining was strongly expressed by cells lining irregular vascular spaces and by tumor cells. In angiosarcoma of other sites, the staining rates were as follow: 5/12 (42%) for D2-40, 9/12 (75%) for endoglin, 10/12 (83%) for CD34, 12/12 (100%) for CD31, respectively. There was no difference in the staining pattern between the two groups. D2-40 and endoglin were negative in hemangioma group (0%), while CD31 and CD34 were positive in all cases (100%).

Conclusions: Our findings showed that angiosarcomas arising after radiation therapy for breast cancer express D2-40, a lymphatic marker, suggesting that they are derived from a lymphatic lineage. In addition, endoglin (a specific marker for proliferating microvessels), was positive in a small percentage of these tumors, raising the possibility of dual origin. Of interest, a high percentage of angiosarcomas arising from other sites were negative for D2-40 and positive for endoglin, supporting their histogenesis from blood vessels.

111 Is Excision of All Radial Scars Diagnosed in Needle Core Biopsies Necessary?

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Background: There is still debate in the literature whether it is mandatory to excise all radial scars diagnosed in needle core biopsies. At our centre, there is also controversy in the management of these cases

Design: The objective is to correlate the needle core biopsy diagnosis with subsequent excisional biopsy and/or clinical/radiological follow-up to determine whether excision for all radial scars diagnosed in needle core biopsies is justified. This is a retrospective study in which 27 cases were retrieved from the surgical database at our centre with histological diagnosis of radial scar on needle core biopsies from the years 1995 to

2005. The slides of the needle core biopsies were reviewed by a breast pathologist without the knowledge of subsequent excisional biopsy and/or radiologic-clinical follow-up and were classified as radial scar with and without atypical hyperplasia. We used Page et al criteria for the diagnosis of atypical ductal and lobular hyperplasia. Radiologic films were reviewed by the breast radiologist without the knowledge of subsequent excisional biopsy diagnosis and the radial scar lesions were classified as classical or nonclassical. Excisional biopsies were reviewed by another breast pathologist and occurrences of carcinoma in subsequent excisions were noted. Mammographic and clinical follow-up was obtained for all the cases which did not undergo excisional biopsy.

Results: 27 cases of radial scar were diagnosed on needle core biopsy. 4/27 cases had the diagnosis of radial scar with atypical hyperplasia and 3/4 had a subsequent excisional biopsy which revealed carcinoma in one case only and 1 case did not show any new lesion on radiologic follow-up. Of the remaining 23 cases with the diagnosis of radial scar without atypia, 9 had excisional biopsy none of which showed evidence of carcinoma. 14/23 cases did not develop subsequent carcinoma on clinical/radiologic follow-up.

Conclusions: Radial scars with no atypical features need not be excised after needle core biopsy and can be safely followed-up clinically and radiologically.

112 Molecular Evaluation of Multiple Breast Carcinomas: Ipsilateral and Contralateral Metastasis vs. Separate Primaries

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Background: In patients with multiple breast carcinomas (CAs), the distinction between separate primaries versus a metastasis can have prognostic and therapeutic implications. Histology is limited in making this distinction, especially if the CAs are of the same histology type. We evaluated the efficacy of molecular analysis of synchronous and metachronous breast carcinomas for differentiating two separate primaries versus a breast metastasis.

Design: Paraffin blocks were retrieved from 48 invasive CAs from 24 patients, including 18 synchronous ipsilateral, 4 synchronous bilateral, 14 metachronous ipsilateral and 12 metachronous bilateral. The CAs in each patient were the same histologic type, or the same type in a mixed tumor. There were 42 ductal carcinomas, 4 lobular carcinomas and 2 mixed ductal-lobular.DNA from neoplasm and non-neoplastic controls removed under stereoscopic guidance was analized by PCR for LOH at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q and point mutation determination in k-ras-2 . These markers were chosen based on molecular abnormalities reported in the literature and on preliminary data from our laboratory. The CAs were classified as de novo or metastasis based on 3 levels of concordance: (1) marker affected - CAs were considered concordant if 50% or more of the same markers were mutated, (2) same gene copy affected, and (3) temporal sequence of mutation. We reviewed the H and E slides for the following: special type, nuclear grade, tubule formation, and subjective impression.

Results: Molecular results are showed in the table below.

	De Novo	Metastases
Synchronous ipsilateral	6	3
Synchronous bilateral	2	0
Metachronous ipsilateral	7	0
Metachronous bilateral	6	0

1/3 metastatic CAs was considered similar by histology. 14/21 de novo tumors were similar by histology. There was no significant difference between different histologic types for de novo and metastases.

Conclusions: Most multiple breast carcinomas were de novo tumors by molecular analysis, suggesting a "field effect". Histology can't reliably make the distinction between de novo tumors and metastasis. The 3/24 carcinomas that were metastatic were synchronous ipsilateral, supporting intraductal spread as mechanism of multiple carcinomas. All the metachronous carcinomas were de novo, vs. 8/11 synchronous. The majority of multiple breast carcinomas are de novo. When breast metastases occur at presentation, they involve the same breast.

113 Invasive Micropapillary Carcinoma of Breast: Association of Pathologic Features with Lymph Node Metastasis

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Background: Invasive micropapillary carcinoma (IMPC) of breast is a WHO recently recognized subtype of epithelial tumors with a high incidence of axillary lymph node metastasis. Pathologic features associated with the lymph node metastasis have not been studied.

Design: To investigate the relationship between pathologic features and lymph node metastasis, fifty-one cases of IMPC of breast were selected from the archive of our institution for microscopic examination and for correlation with the status of axillary lymph nodes. Immunohistochemistry for VEGF-C and VEGFR-3 was performed and lymphatic vessel density was measured.

Results: It was found that: (1) The number of lymph nodes with metastasis in the group of IMPC with histologic grades II/III (mean 12.5) was significantly higher than that in the group with histologic grade I (mean 4.0) ($P<0.05$); (2) The incidence of lymph nodal metastasis was increased significantly in the group with prominent (+/++) lymphocyte infiltration (96.4%) than that in the group with no or minimal (–/±) lymphocyte infiltrates (60.9%)($P<0.005$), and more lymph nodes with metastasis per case was noted in the former group (mean 14.4) ($P<0.005$); (3) VEGF-C expression was in positive correlation with increasing histologic grades ($P<0.05$), lymph node metastasis ($P<0.05$) and lymphatic vessel density ($P<0.01$). IMPC with histologic grades II or III showed more expression of VEGF-C than those with histologic grade I ($P<0.05$, $P<0.01$ respectively). More lymphatic vessels (density) were identified in cases with lymph node metastasis ($P<0.01$); (4) Percentage of IMPC in the tumor was not associated with the incidence of lymph node metastasis. The metastatic foci in lymph nodes were either pure or predominant micropapillary carcinoma. (5) In fourteen of the twenty-eight cases with ductal carcinoma in situ (DCIS), the DCIS was micropapillary type.

Conclusions: The results suggest that the histologic grade, lymphatic vessel density and lymphocyte infiltration of IMPC be the key factors that influence its lymph node metastasis, and certain histologic features might predict lymph node metastasis. Overexpression of VEGF-C and VEGFR-3 facilitates lymph node metastasis. Micropapillary type of DCIS might represent the early stage of IMPC.

114 Pure Apocrine Carcinomas of the Breast Are Characterized by Consistent Aneuploidy, High Proliferative Fraction and Overexpression of Her-2 and p53

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Background: Pure apocrine carcinomas (PAC) are defined as tumors composed entirely of epithelium showing apocrine differentiation (large cells with prominent eosinophilic, flocculent cytoplasm with sharply defined borders and large nucleus with prominent macronucleolus). A recent study has shown that PAC may have a better prognosis than poorly differentiated invasive ductal carcinomas, which contradicted some previous observations. However, none of these studies addressed the characteristic genetic expression / immunophenotypic profile necessary for diagnosis of apocrine cells (androgen receptor positive, GCDFP-15 positive, ER(alpha) negative and PR negative). We investigated several prognostic markers in such strictly defined subtype of mammary carcinoma.

Design: 30 carcinomas that were previously considered apocrine type based on routine H&E histologic appearances were investigated for the expression of apocrine markers using routine immunohistochemical methods. Cases fulfilling those criteria were further evaluated for the DNA content, S-phase fraction, proliferative fraction (MIB-1), Her-2 and p53 expression using flow cytometric, image analysis and immunohistochemical methods.

Results: Twenty out of 30 cases fulfilled immunophenotypic criteria for inclusion in PAC category. All PAC cases then showed aneuploid DNA pattern (average D.I±S.D. = 1.81 ± 0.43), high S-phase (5.45% ± 2.8%) and moderately elevated MIB-1 index (18.7%). Her-2 was overexpressed in majority of cases (70% with 3+ score). Similarly, p53 overexpression (>20% positive cells) was seen in 85% of cases

Conclusions: Immunophenotypic evaluation of invasive carcinomas with apocrine-like morphology is necessary to strictly define "pure apocrine carcinomas". Such defined tumors show features of high grade, aggressive carcinomas associated with expression of many negative prognostic markers. Future studies in clinical outcomes must involve strict definitions of PAC in order to achieve desired goals of separating this subtype from other high grade carcinomas.

115 Immunohistochemical Characterization of Subtypes of Male Breast Carcinoma

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Background: Male breast cancer accounts for less than 1% of all breast cancer and has a rising incidence and worse prognosis than its female counterparts. Recent DNA microarray studies on female breast cancer have identified distinct subtypes that are associated with different clinical outcomes, with poor prognosis seen in HER2 and basal-like subtypes. In addition, epidermal growth factor receptor (EGFR) is over-expressed in aggressive breast cancer and currently evaluated in clinical trail as a potential therapeutic target. Despite these advances, our understanding and treatment strategies for male breast cancer are limited and generally extrapolated from our knowledge of female breast cancer. The current study is aimed to identify the subtypes of male breast cancer based on expression profile of tumor cells and evaluate their association with EGFR expression.

Design: Forty-two cases of male breast cancer were selected from the surgical pathology archives from 1997 to 2005. The study included 39 cases of invasive ductal carcinoma and 3 cases of invasive papillary carcinoma. Ages ranged from 33 to 87 years with a median age of 64 years. Tissue sections from these cases were stained with antibodies against ER, PR, HER2, CK 5/6 and EGFR. Immunostains were then interpreted by two pathologists independently.

Results: Male breast carcinoma showed high expression rates for ER, PR and HER2 (Table1). Compared to published data in female, our group of male breast cancer consisted of higher percentage of luminal (48.8) and HER2 subtypes (47.6%) with rare basal-like (2.4%) and no negative subtypes (0%). EGFR expression was observed to be closely associated with HER2 subtype (78.6%) (Table2).

Conclusions: In our study group, luminal and HER2 subtypes are the most common types in male breast carcinoma. Basal-like and negative subtypes are rare. EGFR expression is closely associated with HER2 subtype of male breast carcinoma.

Table1: Immunostaining results in male breast cancer (n=42)

Antigen	%, (positive cases)
ER	92.1 % (39)
PR	69.1 % (29)
HER2	47.6 % (20)
CK5/6	33.3 % (14)
EGFR	33.3 % (14)

Table2: Male breast carcinoma subtypes and their association with EGFR expression

Subtype	Cases (%)	Immunoprofile	EGFR + (%)
HER2	20 (47.6)	ER any, Her2 ++-+++	11 (78.6)
Luminal	21 (48.8)	ER +, Her2 -	3 (21.4)
Basal-like	1 (2.4)	ER -, Her2 -, CK 5/6 +	0
Negative	0	All negative	0
Total	42 (100%)		14 (100%)

116 Correlation of DNA Mismatch Repair Genes, Hormone Receptor Status and Proliferation Markers in Male Breast Cancer

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Background: Male breast cancer (MBC) is a rare disease, comprising only about 1% of breast cancers. There is relatively little known about the etiology of MBC, and of its relation to female breast cancer. It has been proposed that there is an increased risk for MBC in men with BRCA2 or androgen receptor (AR) germline mutations. A significant number of MBC cases are positive for estrogen (ER) and progesterone (PR) receptors. The reported absence of prostate specific antigen (PSA) in MBC is indicative of a primary androgen-regulated carcinoma rather than metastatic disease. Her2/Neu positivity has been observed as a distinguishing feature of MBC which has been shown to be inversely correlated with that of ER. DNA mismatch and DNA repair genes play a role in the pathogenesis of some carcinomas; eg. colorectal and female breast carcinomas. However, all these factors have not been examined in MBC.

Design: We constructed a tissue microarray comprising of cores from archival formalin fixed paraffin embedded samples from 15 MBC patients (median age 63.5). Immunohistochemistry for AR, ER, PR, Her2/NEU, the DNA mismatch repair (MMR) genes MSH2 and MLH1, PSA, and DNA repair proteins BRCA1 and BRCA2 were performed on an automated immunostainer.

Results: All of the tumors examined were PSA negative. None of the cases had a loss of the MMR genes MSH2 or MLH1. PR showed no significant correlation with MBC. In contrast, ER expression was high in a significant number of tumors (12/14). Interestingly, >50% of tumors had high levels of Her2/Neu protein, without any inverse correlation with ER status. All of the tumors exhibited high expression of AR, similar to published reports. Both BRCA1 and BRCA2 staining was high in all of the tumors. All of the samples had strong nuclear staining for BRCA1with only 10/14 showing nuclear and cytoplasmic localization. Interestingly, BRCA2 staining was predominantly cytoplasmic (3/14 patients had more intense cytoplasmic staining than nuclear) or exclusively cytoplasmic (50% of tumors).

Conclusions: MBC tumors exhibit high levels of AR and ER. No inverse relationship between ER and Her2/Neu expression was found. MMR proteins MLH1 and MSH2 were present at normal levels, suggesting that they do not play any significant role in sporadic MBC. However, while BRCA1 exhibited a nuclear localization pattern, the majority of BRCA2 was localized to the cytoplasm, practically inactive as it is inaccessible for DNA repair.

117 Molecular Markers in Usual Ductal Hyperplasias from Aesthetic Mammaplasty and Invasive Mammary Carcinoma Specimens

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Background: Molecular evidences suggest a number of different pathways leading to development of invasive mammary carcinoma (IMC). The multistep model of breast carcinogenesis suggests a transition from normal epithelium to invasive carcinoma via non-atypical hyperplasia, atypical hyperplasia, and in situ carcinoma. The biological impact of cell cycle regulatory proteins on breast cancer progression is widely recognized, although mostly unclear. The aim of this study was to investigate the correlations of cell-cycle modulators (p53 and p21), p63, estrogen and progesterone-receptors, and a cell proliferation marker (Ki-67) by immunohistochemistry (IHC) in usual ductal hyperplasia (UDH) of patients with and without IMC.

Design: Sections obtained from formalin-fixed, paraffin-embedded tissue from 33 aesthetic mammaplasty and 31 IMC specimens were submitted to IHC using the streptavidin-biotin-peroxidase method. Primary monoclonal antibodies against estrogen receptor(ER), progesterone receptor (PR), p53, Ki-67, p21 (waf-1), and p63 were used (Table 1). Stainings were evaluated in usual hyperplasias of aesthetic mammaplasties (UDH-AM) and in usual hyperplasias adjacent to IMC (UDH-IMC), and in the tumoral cells for the different antibodies using cut-offs described in literature.

Results: Results are summarized in Table 1. There was no significant difference of the molecular markers expression in hyperplasias of both types of specimens. However, the number of positive cells for ER and PR was significantly higher in hyperplasias compared to cancer cells. p53 and p21 were expressed only in cancer cells. Both types of UDH showed lower Ki-67 expression than IMC

Conclusions: Our results demonstrated that the pattern of immunoexpression of molecular markers in UDH from mammaplasty and breast cancer specimens was similar. These results support the concept that UDH is unproven as deterministic precursor lesions, but a marker of slightly elevated breast cancer risk.

Percentage of positive cells for molecular markers in usual ductal hyperplasias of aesthetic mammaplasty (UDH-AM) and adjacent to IMC (UDH-IMC) and in IMC cells

		Lesion Scored		
Antibody	Clone	UDH-AM	UDH-IMC	IMC cells
ER	6F11	90.9%	93.5%	54.8%
PR	PgR 312	100%	96.8%	54.8%
p53	DO7	0 %	0 %	54.8%
p21 (waf-1)	4D10	0%	0%	54.8%)
p63*	4A4	100%	93.5%	6.5%
Ki-67**	Mib-1	low= 90.9%; mod=9.1%	low= 93.5%; mod= 6.5%	low=22.6%; mod=54.8%; high=22.6%

*p63 scored in the myoepithelial cells and tumoral cells; ** Ki-67 scored as low, moderate (mod) and high proliferative rate

118 A Comparative Study between New Rabbit Monoclonal Antibodies and Mouse Monoclonal Antibodies Anti-Estrogen Receptor in Breast Cancer

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Background: Estrogen receptor (ER) has been extensively validated as a predictive factor in breast cancer and immunohistochemistry (IHQ) is now the standard for assessment of ER status. Rabbit monoclonal antibodies (RabMab) represent a new category of immunoreagents that may combine the properties of both mouse monoclonal antibodies (Mab) and rabbit antibodies. We compared the sensitivity of 2 new RabMabs with 3 Mabs, from 4 different suppliers, for the evaluation of ER in breast cancer.

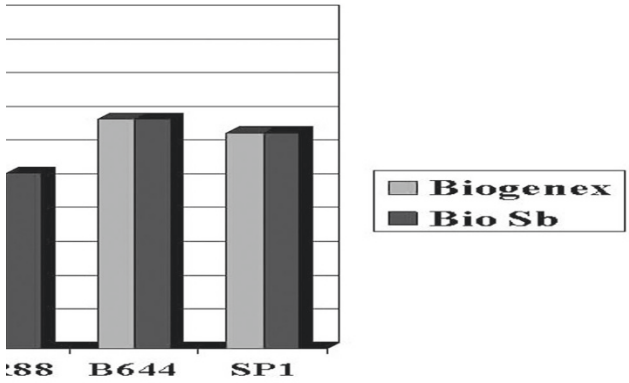
Design: A tissue microarray (TMA) containing duplicate 2mm cores from 25 invasive breast cancers was built. Serial sections were obtained and submitted to IHQ using two different streptavidin-biotin detection systems (BioSB and Biogenex) and 6 anti-ER monoclonal antibodies: 2 RabMab (SP1, LabVision and B644, BioSb) and 4 Mab (1D5, Dakocytomation; 1D5, BioSB; 6F11,Novocastra; ER88, Biogenex). Antibody dilutions and antigen retrieval were used following the supplier instructions. TMA were double-tested for each antibody resulting in a total of 24 slides with 1200 immunostained tissue cores. Slides were scored blindly by two pathologists and considered positive when >10% of tumor cell nuclei stained for ER.

Results: The results are summarized in Table 1 and Fig. 1. Higher positivity rates were obtained with RabMab ER-antibodies compared to Mab antibodies, regardless of the detection system used ($p<0.01$). Only the 6F11 Mab showed similar levels of positivity obtained with the RabMab.

Conclusions: Our data showed that the RabMab anti-ER antibodies (SP1 and B644) are significantly more sensitive to identify positive ER tumors compared to Mab 1D5 and ER88, but similar to 6F11. Rabbit monoclonal antibodies may improve identification of ER-positive breast cancers.

Percentage of ER-positive breast cancers comparing different RabMab and Mab antibodies

	Detection System	
Antibody	Biogenex	Bio SB
1D5 (Dakocytomation)- Mab	60%	40%
1D5 (Bio SB)- Mab	56%	48%
6F11 (Novocastra)- Mab	68%	64%
ER88 (Biogenex)- Mab	48%	52%
B644 (Bio SB)- RabMab	68%	68%
SP1 (Labvision)- RAbMab	64%	64%
Total	100%	100%



119 Most Triple-Negative Invasive Breast Cancers Express a Basal/Myoepithelial Phenotype

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Background: About 20% of breast carcinomas are negative for estrogen receptor (ER), progesterone receptor (PR) and HER2 (triple-negative). Included among this group are tumors morphologically consistent with metaplastic and medullary-type carcinomas of the breast, as well as high grade mammary carcinomas of no special type. A basal/myoepithelial phenotype has been suggested for these triple-negative breast carcinomas. Overlapping morphologic characteristics, including a solid growth pattern and high nuclear grade, may preclude accurate classification of these tumors, particularly in core biopsies. We evaluated the use of p63 — expressed by myoepithelial/basal cells, P-Cadherin — expressed by myoepithelial/stem cells, and HLA-DR — a Class II MHC antigen, as potential immunohistochemical markers for the subclassification of triple-negative mammary carcinomas.

Design: One hundred and twenty-two formalin-fixed, paraffin embedded cases of ER, PR, and HER2 negative breast carcinomas were morphologically classified according to WHO criteria, and evaluated for the immunohistochemical expression of p63 (DakoCytomation), P-Cadherin (Novocastra), and HLA-DR (BioGenex) using the L-SAB detection system.

Results: Cytoplasmic membrane immunostaining for P-Cadherin was present in 40 of 45 cases of metaplastic carcinoma, 25 of 27 medullary carcinomas, and 44 of 50 poorly differentiated non-medullary, non-metaplastic mammary carcinomas. P63 was preferentially expressed in the group of metaplastic carcinomas, with positive nuclear immunostaining present in 43 of 45 cases. All medullary-type carcinomas demonstrated a positive immunoreaction of the cytoplasmic membrane for HLA-DR. Of the tumors morphologically classified as poorly differentiated carcinomas of no special type, 11 (22%) were positive for p63 and/or HLA-DR.

Conclusions: A significant proportion of breast carcinomas that are negative for ER, PR, and HER2 express a basal/myoepithelial/stem cell phenotype. Metaplastic,

medullary, and poorly differentiated mammary carcinomas of no special type are included in this group and may be histogenetically distinct from the usual ductal/lobular phenotypes. The combination of p63, P-Cadherin, and HLA-DR may assist in the subclassification of these triple-negative cases, particularly when sufficient morphologic characteristics may be lacking.

120 Significant Incidental Pathologic Findings in Reduction Mammoplasty Cases: A Five-Year Review

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Background: Reduction mammoplasty (RMP) is performed for macromastia, congenital asymmetry or asymmetry following breast cancer surgery. The incidence of incidental breast cancer in RMP specimens is reportedly 0.06% in women without prior cancer and up to 4.6% in women with contralateral breast cancer. No standard pathology assessment for RMP specimens exists; some institutions evaluate the same initial number of tissue blocks, whereas others examine more blocks in older women or in those with contralateral breast cancer. This study determined the incidence of significant incidental pathologic findings in RMP cases in order to evaluate age as a risk factor for proliferative breast disease. This evaluation will help formulate guidelines for pathologic evaluation of RMP specimens.

Design: A retrospective review of RMP cases in women of any age who underwent unilateral or bilateral RMP for any reason from January 2000 to May 2005 was undertaken. All patients had similar sampling regardless of age. RMP cases with ipsilateral breast cancer were excluded. Patient age, specimen weight, and pathologic diagnoses were recorded. Archival slides with significant pathologic findings, including moderate ductal hyperplasia, atypical ductal or lobular hyperplasia, ductal or lobular carcinoma in situ (DCIS, LCIS), and invasive carcinoma were reviewed. Cases were grouped into non-proliferative disease (non-PD), proliferative disease without atypia (PDWA), and proliferative disease with atypia or cancer (PDAC).

Results: The study included 552 cases. 85 (15.4%) had proliferative disease; of those, 75 (88.2%) were PDWA and 10 (11.8%) were PDAC. The mean age of women with and without proliferative disease was 47.4 and 37.7 years, respectively ($p<0.001$;t-test analysis). Women with PDAC were older compared to those with non-PD (50.7 versus 37.7 years, respectively; $p=0.0009$; Wilcoxon rank sum analysis). Age was not significant when comparing PDAC to PDWA ($p=0.33$). The cancer rate was 0.9% (2-LCIS, 2-DCIS, 1-invasive lobular).

Conclusions: Age was associated with proliferative breast disease compared with non-proliferative breast disease in identically sampled specimens. Age was also associated with proliferative breast disease with atypia or carcinoma compared with non-proliferative disease. A prospective study addressing whether increased sampling yields higher rates of significant incidental proliferative breast disease in women of any age or risk category is ongoing.

121 Expression of Claudin 7, ZO-1 and SOX4 in Florid Hyperplasia and Breast Carcinoma

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Background: The presence of florid hyperplasia without atypia (FHWA) in a benign breast biopsy is associated with a moderate risk of subsequent invasive mammary cancer (IMC); however, molecular markers linking subsets of at risk patients to the histologically observed architectural alterations in orientation and polarity are needed. Claudin-7 (C7), a tight junction transmembrane protein, is linked to a cytoplasmic adapter protein ZO-1, anchoring C7 to cytoplasmic and cytoskeletal elements and recruiting regulatory and signaling molecules. SOX-4 is a progestin regulated gene whose normal expression plays a role in the differentiated mammary gland. Kominsky (*Oncogene, 2003)* demonstrated an inverse correlation between C7 expression and histological grade in both ductal carcinoma in situ (DCIS) and IMC. Similarly, Martin (*EJC, 2004*) reported decreased intensity of ZO-1 staining by IHC and expression by western blot analysis with increasing IMC grade. Graham (J Mol Endo, 1999) has detected higher levels of SOX-4 expression in IMC compared to normal breast. Aberrant expression/localization of these molecules in FHWA may reflect significant deviations from the normal orientation and polarity or response to progestins.

Design: Localization and intensity of membranous C7 & ZO-1 and nuclear SOX-4 expression were examined by IHC in 31 IMC, 14 DCIS, 7 FHWA, 2 enlarged lobular units with columnar alteration (ELUCA), 5 papillary apocrine change (PAC), and 56 normal lobular units (NLU). Intensity of staining was graded from 0 to 3+.

Results: ELUCA, PAC, and NLU demonstrated basolateral and apical 2-3+ staining of epithelial cells by C7 and ZO-1, respectively. The intensity of C7 and ZO-1 were decreased in DCIS and IMC but only C7 was inversely related to histological grade. Characteristic apical ZO-1 staining was maintained in tumor lumena but showed circumferential distribution and focal loss in solid areas of tumor and FHWA. FHWA showed 2+ basolateral C7 staining peripherally and focal to complete loss centrally. SOX-4 exhibited weak to moderate nuclear staining in all groups.

Conclusions: Aberrant C7 and ZO-1 localization and expression loss in FHWA and IMC reflect significant deviation from the normal orientation and polarity of mammary epithelium in these processes. Maintenance of normal ZO-1 and C7distribution in tubular carcinoma reinforces the very low malignant potential of this special type of IMC. Larger studies correlating C7 and ZO-1 expression in FHWA with outcome may serve to link these architectural alterations with neoplastic transformation.

122 Significant Loss of PTEN Protein Expression in Lymph Node Metastases of Primary Breast Cancer: An Immunohistochemical Study Using Tissue Microarray

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Background: The tumor suppressor gene PTEN encodes a dual phosphatase that plays a significant role in cell-cycle regulation, apoptosis, cell adhesion, and cell differentiation. In an earlier study, we found that PTEN activation makes breast cancer cells more responsive to HER-2-targeting antibody therapy with trastuzumab and that patients with PTEN-deficient breast cancers have a poor response to the therapy (Nagata Y, et al. Cancer Cell 6:117-127, 2004). Therefore, evaluation of PTEN has clinical significance for patients who are being considered for trastuzumab therapy. Unfortunately, there is not yet a standard method for PTEN evaluation in clinical practice. Immunohistochemistry has been used to detect PTEN expression. However, whether this expression is homogeneous or heterogeneous in primary tumors and metastases has not been extensively studied. In this study, we examined the expression of PTEN in primary breast cancers and matched lymph node metastases using tissue microarray, which allows simultaneous assessment of tumor marker expression in a large number of samples.

Design: Invasive primary breast cancer specimens and corresponding axillary lymph node metastases from 67 patients were obtained. Three 1-mm cores were taken from each specimen. Immunohistochemistry for PTEN was performed using Ab-2 polyclonal antibody (Neomarkers). The staining intensity (in a scale of 0-3) and positivity percentage (in a scale of 0-5) were assessed visually using the Allred scoring system. The sum of these scores that ranges 0-8 was used as PTEN score. Correlation of PTEN scores of primary carcinoma and lymph node metastases was evaluated. All statistical tests were performed using SAS version 8.02 at a significance level of 5%.

Results: PTEN expression scores of lymph node metastases were significantly lower than those of primary tumors ($p<.0001$). The median PTEN score was 6 (range 3-8) in the primary tumors and 5 (range 2-7) in the lymph node metastases. PTEN expression of primary tumor and lymph node metastases were uniform without regional differences.

Conclusions: Our findings support the hypothesis that lower PTEN expression is associated with metastatic phenotype in breast cancer. Development of a standard quantitative assay for PTEN evaluation is necessary for clinical use. Metastatic tumor samples are more informative for assessment of PTEN protein levels than primary tumor samples.

123 Intratumoral Heterogeneity of HER2/*neu* in Breast Cancer – A Rare Event or an Overlooked Phenomenon?

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Background: Her2/*neu* is, overexpressed in about 20% of invasive breast carcinomas. Numerous studies have shown that there is high level of concordance between the Her2/*neu* status of the primary breast cancer and the metastases of a given patient. Recently changes in Her2/*neu* status with tumor progression have been reported, suggesting the possibility of an emerging different tumor clone. Little is known about intratumoral heterogeneity with regard to Her2/*neu* oncoprotein overexpression.

Design: We examined 9 /921(1%) cases of primary invasive breast carcinomas that showed heterogenous immunohistochemical staining that were identified in a 6 months period. We compared the results of the IHC to both FISH and CISH and assessed the morphologic characteristics and hormone receptor status in relation to Her2/*neu* expression.

Results: All 9 tumors were invasive ductal carcinomas (not otherwise specified) 8 with DCIS component. These cases had a heterogenous pattern of staining, in 6 cases the tumors were predominantly negative, and in 3 cases the tumors were predominantly positive for HER2/neu overexpression. In all cases the areas of overexpression noted on immunohistochemistry were associated with gene amplification in the corresponding area of the tumor by both methods of in situ hybridization (CISH and FISH). The variation in HER2 status was accompanied by changes in architecture(3), ER(1), PR(1).

Conclusions: The results of this study suggest that in some cases of breast carcinoma there is a clonal selection resulting in focal areas showing loss or amplification of the HER2 gene in a primary tumor that showed variability of Her2/*neu* oncoprotein overexpression. Heterogeneity of Her2/*neu* status in a tumor may be a rare event or an overlooked phenomenon. This may represent tumor heterogeneity of the non-cancer producing progeny or an incidental marker of the clonogenic tumor initiating cancer cells that supports the hierarchal theory of carcinogenesis. This phenomenon should be examined since it may contribute to a better understanding of the variation in therapeutic responses and the conflicting data in studies about the prognostic and predictive role of Her2/*neu* status in subsets of breast cancer patients.

124 Expression of the Undifferentiated Cell Marker P63 in Primary Invasive Carcinomas of the Breast and Their Nodal Metastases

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Background: Although lymph node negative breast cancer patients have a favorable outcome, once lymph node metastasis develop the survival decreases significantly. Undifferentiated pluripotential stem cells have been shown to give rise to neoplasias. A current hypothesis is that a subset of undifferentiated stem cells in the breast are the cell type most capable of establishing a metastatic deposit. In addition to being expressed in myoepithelial cells, P63 marks undifferentiated stem cells. The current study seeks to investigate the presence of P63 reactive undifferentiated cells in primary invasive carcinomas and their lymph node metastasis.

Design: Twenty primary invasive carcinomas of the breast and their corresponding 20 nodal metastasis were studied histologically and were immunostained using anti-P63 and anti-muscle specific actin (MSA) antibodies. Internal positive and negative control cells including squamous epithelial, stromal, and benign breast tissue adjacent to

tumor were present in all cases. P63 immunostaining was considered positive when any malignant cell showed nuclear staining. MSA was used primarily to control that the P63 positive cells were not myoepithelial cells.
Results: Of the 20 primary invasive carcinomas, 18 were invasive ductal carcinoma, 1 had ductal and lobular features, and 2 were invasive lobular carcinoma. None of the tumors had squamous metaplasia. Immunoreactivity for P63 was identified in 14 out of 20 (70%) of the primary tumors and in 10 out of 20 (50%) of the nodal metastases. Nuclear staining for P63 was found within less than 5% of malignant cells in most cases. Three (17%) of the nodal metastases of invasive ductal carcinomas and 2 (11%) of the corresponding primary tumors showed widespread nuclear staining (over 50% of the cells). The P63 positive cancer cells were negative for MSA, supporting their lack of myoepithelial differentiation.
Conclusions: The majority of primary invasive carcinomas with metastasis contain at least a small percentage of P63 positive undifferentiated cancer cells. A subset of these primary tumors shows widespread reactivity. P63 positive undifferentiated cancer cells are also detected in the lymph node metastasis. Taken together, these data support the theory that stem cells contribute to the metastatic potential of breast cancer. Although they only make up a small fraction of cells in most of the tumors, it is plausible that a small number of pluripotential stem cells may seed the growth of cancer in the nodes.

125 Ezrin, a Cytoskeletal Linker Protein Is Expressed in a Subset of Breast Cancers and Correlates with Estrogen Receptor Expression and a More Favorable Clinical Outcome

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Background: Ezrin, an intracellular protein that facilitates linkage between the cell membrane and the cytoskeleton, provides scaffolding that allows the cell to interact with its microenvironment. As a result, ezrin facilitates a number of cellular functions and signal transduction pathways, which may be altered and affect the clinical behavior of certain types of malignancy. Ezrin over-expression has been correlated with poor outcome in a number of mesenchymal tumors, including osteosarcoma and soft tissue sarcoma. However epithelial tumors, such as serous ovarian tumors, show a worse clinical outcome when ezrin expression is lost. In this study, we investigated the prognostic significance of ezrin expression in breast cancer.
Design: Tissue microarrays were constructed containing 268 primary breast cancers with a mean clinical follow-up time of 90 months. Ezrin expression was determined by immunohistochemical staining (1:25, BD Transduction Laboratories), scored on a 0-3+ scale and compared with established prognostic and predictive factors.
Results: Immunoreactivity for ezrin was seen in 44.8% of breast tumors (120/268) and demonstrated either a granular cytoplasmic or membranous pattern of staining. Ezrin expression correlated with ER positivity (p=0.002) and a lower grade (p=0.019), particularly when the staining was restricted to the cell membrane (p=0.001). Ezrin expression also correlated with an improved disease free survival (DFS) (p=0.03), which was not independent of grade and ER status.
Conclusions: The reported results suggest that ezrin expression in breast cancer is associated with ER positivity and improved DFS. By linking events at the cell surface with the cytoskeleton, ezrin plays an important role in a number of cellular functions, including cell adhesion, matrix attachment, cell survival and motility. Expression of ezrin has been correlated with a poor clinical outcome in a number of tumors leading to the hypothesis that ezrin may be a nexus of metastasis. Our results suggest that ezrin could have opposing effects on the clinical course of different types of tumors, depending on the context and the cell type in which it is expressed. Further study of ezrin's role in the tumor biology and clinical behavior of breast cancer may be warranted.

126 Breast Cancers with Brain Metastases Are More Likely To Be Estrogen Receptor Negative, Express the Basal Cytokeratin CK5/6 and Over-Express HER2 and EGFR

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Background: Brain metastases (BM) remain a devastating complication of breast cancer and are associated with significant morbidity and poor survival. The incidence of BM appears to be increasing, which is likely due to better treatment regimens and longer survival times. In the current study we have examined a cohort of breast cancer patients who went on to develop BM for clinical-pathologic features and predictive markers that identify this high risk subgroup of patients at the time of diagnosis.
Design: The primary tumors from 74 patients who developed BM were used to construct a tissue microarray (TMA). The clinical and pathologic features were recorded and the TMA was stained for ER, HER2, CK5/6, and EGFR by IHC. This cohort of patients was compared against a group of 258 patients who remain free of metastases at 8 years (NM), and another cohort of 64 patients who developed mixed visceral and bone metastastic disease (VM) without brain recurrence over a similar time period. HER2 and EGFR genotype was also correlated with protein expression.
Results: Breast cancer patients who went on to develop BM were more likely to be <50 years old compared with NM (p<0.001) and VM (p=0.17), and the primary tumors were more likely to be ER negative (p<0.001) and high grade (p=0.002). The primary tumors from patients who developed BM were more likely to express CK 5/6 (p<0.001)and EGFR (p<0.001), and to over-express HER2 (p<0.001). HER2 IHC over-expression showed a good correlation with gene amplification, while EGFR did not.
Conclusions: This study suggests that breast cancer patients at increased risk for developing BM are more likely to be <50, and have primary tumors that are ER negative, high grade, express the basal cytokeratin CK5/6, and over-express HER2 and EGFR. This profile suggests those tumors of the "basal-like" phenotype and breast cancers in BRCA1 mutation carriers may be at increased risk for BM, given their propensity to express basal cytokeratins and EGFR. Predictive factors to help identify patients with metastatic breast cancer who are at an increased risk for developing CNS recurrence might allow for screening of this population for early detection or the development of targeted strategies for prevention.

127 Sentinel Lymph Node (SLN) Sampling in Patients with Core Biopsy Diagnosis of Ductal Carcinoma *In Situ* (DCIS): UT M. D. Anderson Cancer Center Experience and Future Recommendations

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Background: SLN sampling has been used for staging in selected patients (pts) with core biopsy (CB) diagnosis of DCIS since a significant number of these pts have invasive carcinoma (IC) on excision. However, guidelines for pt selection are not well defined and many pts without IC undergo unnecessary SLN sampling. In this retrospective study, we evaluate pts with DCIS on CB, with the following objectives: 1) to determine the prevalence of SLN metastases; 2) to determine the incidence of IC on excision; and 3) to identify potential pathologic/radiologic predictors of IC to improve selection of pts for SLN sampling.
Design: Two hundred pts with DCIS on CB with excisions were identified in computer database in Pathology of MDACC from 05/2002 to 06/2005. Demographic data, size and type of lesion (mass or calcifications) on imaging, histologic features of DCIS on CB (nuclear grade [NG], pattern, necrosis, lobular cancerization [LC], periductal fibrosis and inflammation), the number of cores taken, and the number of cores involved by DCIS were correlated with IC on excision and SLN outcome.
Results: 1) 103 of 200 pts (52%) had SLN sampling with excision. Prevalence of SLN metastases: 3/103 (3%), with 1 positive SLN each, and size of metastases 6, 1 and 0.5 mm. All 3 pts had IC on excision. Also, 1 pt with DCIS on excison had isolated tumor cells in 1 SLN. 2) Incidence of invasion: 41 of 200 pts (21%) had IC on excision, with size of IC from 0.05 to 1.6 cm. Among the pts with SLN sampling, 34 of 103 had IC (33%). 3) Parameters associated with IC (p values from Chi-square or Fisher's exact tests): mass [14/39 (36%) with mass vs. 27/161 (17%) with calcifications; p=0.008], larger size (median, 2.5 cm vs. 1.5 cm; p=0.001), high NG of DCIS (p=0.03) and presence of LC on CB (p=0.008). A multivariate logistic regression model utilizing all significant parameters from univariate analysis was developed to predict invasion.
Conclusions: The overall prevalence of SLN metastases was 3% and a large number of pts without invasion on excision underwent unnecessary SLN sampling (69/103, 67%). Based on our data, size and type of the lesion, and histologic parameters such as NG and LC are significant predictors of invasion and therefore should be included in the guidelines for the selection of pts for SLN sampling to reduce the number of patients undergoing unnecessary procedure.

128 A Correlation between Primary Breast Tumors Stage T1c and T2 and Presence and Extent of Sentinel Lymph Nodes Involvement

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Background: Axillary lymph node status is the most important prognostic factor in patients with breast cancer. Tumor size and lymph node status are the most important factors that predict survival.
Design: The aim of this study is to identify specific features of primary breast tumors that may predict presence and extent of sentinel node involvement. We examined 83 breast carcinoma cases with positive sentinel lymph nodes during the period January 2004 through June 2005. We included also cases with pN0(i+) and pN1(mi). Sentinel lymph nodes were examined by frozen section and paraffin section as per protocol. We focused our attention on T1cN1 stage and T2 N1 to identify a correlation between primary tumor type, stage, grade, lymphovascular invasion and sentinel lymph node involvement
Results: Out of 83 patients identified 60(72%) had N1stage. The primary tumor had the following distribution 55 invasive ductal carcinoma, 4 lobular carcinoma and 1 ductal carcinoma with lobular features. The tumor stage for N1 was the following 2 T1a, 5 T1b, 22 T1c, 22 T2, 8 T3 and 1 T4b. We found 31 cases with lymphovascular invasion, 24 cases without lymphovascular invasion and 5 cases with indeterminate lymphovascular invasion. The primary tumor grade distribution for N1 was the following: 14 cases grade 1, 24 cases grade 2 and 19 cases grade 3. For T1cN1 group we find that 8(36%) cases had lymphovascular invasion, 3(13%) cases were indeterminate for invasion. The tumor grade distribution was 5(22%) cases grade 1, 12(54%) cases grade 2 and 4(18%) cases grade 3. The sentinel lymph node involvement was present with tumor clusters measuring in range from 0.1 to 1.6 cm. and 2(9%) cases had extracapsular extension of the tumor. For T2N1 group we find that 16(72%) cases had lymphovascular invasion. The tumor grade distribution was 4(18%) cases grade 1, 6(27%) cases grade 2 and 11(50%) cases grade 3. The sentinel lymph node involvement was present with metastasis measuring in range from 0.1 to 2 cm. and 10(45%) cases had extracapsular extension of the tumor. There was no difference in tumor type(ductal versus lobular) between T1c and T2.
Conclusions: We conclude that for primary breast tumors stage T1cN1 and T2N1 there is a strong correlation between tumor grade 3, lymphovascular invasion and sentinel lymph node involvement and especially with extracapsular tumor extension.

129 Magnetic Resonance Imaging (MRI) Guided Breast Core Biopsy: Pathologic Correlation

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Background: Breast MRI is currently being evaluated as an imaging modality for breast cancer. It has especially been recommended for young women with a family history of breast carcinoma and for women with a previous or concurrent history of breast carcinoma. MRI guided mammotome core biopsy is a recently developed technique. We reviewed our experience with MRI directed core biopsy and correlated the cases' pathologic and MRI findings.

Design: Review of our database revealed 44 cases of MRI-guided 9 gauge mammotome core biopsies consisting of 10-12 core specimens. The patients ranged in age from 30 to 77 years (ave=54.2 years). A history of breast carcinoma was present in 27 patients; 20 of which were concurrent, the remaining ranging from 1 to 20 years. Nineteen of these cancers were contralateral. Two patients were premenopausal with a family history of breast carcinoma, 2 patients were previously diagnosed with lobular carcinoma in situ, and 1 with atypical duct hyperplasia. The remaining 12 patients had MRI screening because of dense breasts on mammograms and negative sonograms. MRI indications for biopsy were: enhancing mass = 20, enhancement (ranging from 0.4 to 2.0cm) = 13, irregular linear clumping = 6, indeterminant mass = 4 and a spiculated mass = 1.

Results: Ten out of 44 (23%) cases proved to be malignant, half being intraductal carcinoma (DCIS) and the other half invasive carcinoma (IC) (4 ductal and 1 lobular). Eight out of 10 of these patients had prior or concurrent malignancies, the remaining 2 detected purely due to screening. The remaining diagnoses were as follows: Fibrocystic changes (FCC) = 21, fibroadenoma (FA) = 9, atypical apocrine adenosis (AAA) = 1, intraductal papilloma = 1, duct ectasia = 1, and radial scar = 1. Three out of 5 DCIS cases correlated with irregular linear clumping enhancement. The enhancing masses were diagnosed as follows: FCC = 8, FA = 6, DCIS=2, IC= 3, AAA = 1. The indeterminant masses were equally divided as FA or FCC. The spiculated mass proved to be a radial scar. In the single case of duct ectasia, exuberant surrounding granulation tissue may have caused the enhancement.

Conclusions: The majority of the MRI guided biopsies proved to be benign (77%). The majority of the malignancies were in patients with known other malignancies or other risk factors, thereby decreasing the false positive rate in these situations. With the possible exception of DCIS, no enhancement patterns specific to diagnoses were found. The nonspecific histologic features also make assessment of core adequacy and accuracy problematic.

130 NY-BR-1 – A Novel Differentiation Antigen of the Mammary Gland

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Background: NY-BR-1 was recently identifed by autologous serological typing of breast cancer patients. Extensive RT-PCR analyses revealed NY-BR-1 expression in normal mammary gland, testis and in a high percentage of breast carcinomas. Due to this expression pattern, NY-BR-1 could be an interesting antigen for diagnosis and the immunotherapy of breast cancer. Here we present newly generated mAbs to NY-BR-1 and a preliminary IHC analysis of NY-BR-1.

Design: BALB/c mice were immunized with recombinant NY-BR-1. Newly generated clones were tested for specificity by Western Blotting in transfected and untransfected cell lines, then tested for IHC-reactivity by using various antigen retrieval techniques and finally applied to panels of normal and tumor tissues.

Results: Clones NY-BR-1#2 and #3 were NY-BR-1 specific and used for further analysis. Both mAbs worked well on paraffin tissue using EDTA buffer and heat-based antigen retrieval techniques. In normal tissues, NY-BR-1 protein was present solely in luminal epithelium of ducts and acini of the mammary gland. Staining was cytoplasmic as well as nuclear and mostly heterogeneous. Myoepithelial cells were negative. In breast carcinoma, homogeneous staining was present in the in situ component in 9/11 cases while invasive carcinoma was positive in 5/10 cases. Not surprisingly, sweat gland carcinomas were also focally positive (3/11). Various other carcinomas not related to breast (ovary, colon, NSCLC, pancreas, RCC) as well as melanoma were NY-BR-1 negative.

Conclusions: The protein expression pattern indicates that NY-BR-1 is a true differentiation antigen of the mammary gland. Its high prevalence in normal epithlium, and DCIS and its decreasing expression in invasive carcinoma, speaks for a loss of differentiation during tumor progression. Nevertheless, our data suggest that NY-BR-1is a valuable new diagnostic marker and a potential target for immunotherapy.

131 Clinical Significance of Lobular Neoplasia on Core Biopsy

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Background: A core biopsy diagnosis of atypical ductal epithelial hyperplasia is upstaged on follow-up excisonal biopsy (FUEB) to in situ or invasive carcinoma in approximately twenty-five percent of cases. In contrast, upstaging FUEB information for a core biopsy (cbx) diagnosis of lobular neoplasia (LN), either atypical lobular hyperplasia (ALH) or lobular carcinoma in situ (LCIS) is less clear. In this study, we report the largest retrospective series on a series of patients who had a cbx diagnosis of LN and a FUEB.

Design: The archives of the Dept of Pathology, Magee-Women's Hospital of UPMC was searched from January 2001-August 2005 and yielded a total of 110 cbx from patients who had a cbx dx of LN and who also had a FUEB. Cbx were reviewed and classified as those with pure LCIS, pure ALH or mixed LCIS/ALH. All FUEB were reviewed, and a FUEB was considered upstaged if either/or DCIS or invasive carcinoma was present. Recorded data on pathology reports of radiologic images of all cases was reviewed to determine which cases had calcifications and/or mass.

Results: All cases had calcifications by radiologic imaging, and 5/17 (29%) of the upstaged cases also had a mass on image study. Overall, 15% of patients with a diagnosis of LN on cbx were upstaged on FUEB. Pure ALH had 8/74 (10.8%) cases upstaged, mixed ALH-LCIS had 2/11 (18%) upstaged and pure LCIS had 7/25 (28%) upstaged. If the cases with masses are excluded and those with calcifications only are considered, upstaging percentages are: ALH, 5/74 (6.3%), mixed ALH-LCIS 1/11 (9%), LCIS 6/25 (24%).

Summary LN Upstaging on FUEB

	ALH	ALH+LCIS	LCIS	TOTAL
	8/74 (10.8%)	2/11 (18%)	7/25 (28%)	17/110 (15%)
Mass on Image	3/8	1/2	1/7	5/17

Conclusions: (1) A finding of LN on breast core biopsy is associated with a significant risk of upstaging to a treatable disease on FUEB. (2) The risk of upstaging on FUEB increases in order of ALH, mixed ALH+LCIS and LCIS, whether or not imaging studies show a density or mass. (3) The overall rate of upstaging in this study is somewhat low due to the preponderance of ALH cases.

132 Immunohistochemical Characterization and Prognostic Significance of Basal Type of Invasive Ductal Carcinoma in Breast

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Background: Several studies have shown that gene microarray analysis can recognize clinically significant subsets of tumors. These include the discovery of novel subtype of breast carcinomas, such as Her-2 type, ER type, basal type and negative type. Basal type was characterized by no expression of Her-2 and ER and expression of at least one among EGFR, c-kit and CK 5/6. To evaluate this classification as a prognostic factor of invasive ductal carcinoma, we classified invasive ductal carcinomas into Her-2 type, ER type, basal type and negative type, evaluate survival rate and correlation with TMN stage, histological grade, proliferation index and status of menopause.

Design: The material consisted 295 ductal carcinomas, which were diagnosed at Korea University Guro Hospital between 1992 and 2004. To classify the breast carcinomas, immunohistochemical studies for Her-2, ER, EGFR, c-kit and CK 5/6 were performed using tissue microarray.

Results: Mean period for follow-up was 5.71 years. Mean age of the patients was 44.5years. Her-2 type was 20.7%(61), ER type was 39.7%(117), basal type was 16%(47) and negative type was 23.7%(70). The 5-year overall survival of basal type was lower than ER type (p=0.049). Basal type was related to high-grade carcinomas (p<0.0001). The subtypes did not show any correlation with age, status of menopause, size of tumors, nodal status or proliferation index.

Conclusions: The basal type of invasive ductal carcinoma showed poor prognosis compared to other subtypes of the ductal carcinomas, especially in patients under 35 years and patients with nodal metastasis. This classification can be used for prognostic factors of invasive ductal carcinoma of breast.

133 Clinicopathologic Significance of Basal-Like Subtype in Breast Cancers: Comparison with Hormone Receptor and Her2/neu Overexpressing Phenotypes

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Background: Recent DNA microarray profiling studies have categorized invasive breast carcinomas into luminal/estrogen receptor (ER) positive, normal breast-like, Her2/neu overexpressing and basal-like types. Among them, basal-like carcinoma type has been known to be associated with the worst clinical outcome in Western countries. However, the clinicopathologic characteristics of the basal-like carcinomas compared with other subtypes have not been described in Korean population.

Design: We evaluated the expression of basal CKs (CK5 and 14) and luminal CKs (CK8/18), epidermal growth factor receptor (EGFR), c-kit, hormone receptors (HRs), p53, and Her2/neu in 776 consecutive cases of invasive breast carcinoma from January 1993 to December 1998, using tissue microarray technique, and categorized them into five subtypes (basal-like; HR expressing; Her2/neu overexpressing; both HR and Her2/neu expressing; and null subtypes, all markers negative) based on the immunohistochemical results.

Results: There were 98 (12.6%) cases of basal-like, 345 (44.5%) HR expressing, 133 (17.1%) Her2/neu overexpressing, 61 (7.9%) both HR and Her2/neu expressing, and 139 (17.9%) null subtypes. Histologically, most of the basal-like carcinomas were invasive ductal carcinoma, not otherwise specified (86/98 cases, 87.8%) with high nuclear and/or histologic grades. Most of metaplastic carcinomas (6/8 cases, 75.0%) were basal-like carcinoma. Basal-like carcinomas were associated with larger tumor size (mean, 3.4cm) than HR expressing type (mean, 2.8cm) (p=0.018), and nodal and tumor stages were significantly higher in Her2/neu overexpressing type than basal-like type or HR expressing type (p=0.010 and p=0.045, respectively). Her2/neu overexpressing type also showed distant metastasis most frequently (33.8%), and was the prognostically worst subtype of breast cancers than other subtypes.

Conclusions: Different from the previous studies from Western countries, although basal-like type was an aggressive variant, Her2/neu status appears to be the most important prognostic factor of breast cancers in our study.

134 The Protease Cathepsin K Is Highly Expressed in the Stroma of Human Breast Cancers

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Background: Cathepsin K is the most potent mammalian elastase yet described and possesses unique collagenolytic activity. This enzyme was originally described as being selectively expressed by osteoclasts and studied for its role in osteoporosis. Subsequently cathepsin K was shown to have a pivotal role in lung matrix homeostasis and was noted to be overexpressed in fibroblasts of patients with lung fibrosis. While cathepsins have been studied in matrix remodeling during animal tumorigenesis and are being considered as potential targets in cancer treatment, the expression of cathepsin K in human breast cancer and in pre-invasive breast lesions has not been previously studied.

Design: To address this, immunostains for cathepsin K were performed on tissue microarrays containing quadruplicate tissue cores from 20 examples each of normal

inter- and intra-lobular breast stroma, and stroma from non-proliferative lesions, usual ductal hyperplasia, sclerosing adenosis, radial scar, atypical ductal hyperplasia, non-high grade ductal carcinoma in situ (DCIS), high grade DCIS, and infiltrating ductal carcinomas. Biopsy sites were included for comparison. The tissue cores were evaluated for (1) the proportion of stromal cells stained for cathepsin K and (2) the intensity/pattern of staining (i.e., weak staining with fine punctate pattern or strong staining with coarse punctate pattern).

Results: Cathepsin K expression was observed in fibroblasts in a punctate cytoplasmic distribution, consistent with its lysosomal localization. Whereas the stroma of normal breast tissue and pre-invasive lesions exhibited either no or incomplete staining of the fibroblast population in a fine punctate distribution (seen in >85% of cases), invasive breast cancers and biopsy sites showed cathepsin K expression in the majority of stromal cells, often with a coarse and intense cytoplasmic distribution pattern (seen in >90% of cases). Both benign and malignant breast epithelial cells were predominantly negative for cathepsin K.

Conclusions: These results demonstrate for the first time that cathepsin K is highly expressed in stromal cells of human breast cancers, suggesting that this enzyme may be an important factor in extracellular matrix remodeling and the facilitation of invasion in these tumors. Furthermore, the presence of strong cathepsin K expression in stromal cells of both invasive breast cancers and biopsy sites represents yet another similarity between the processes of tumor stroma generation and wound healing.

135 Significance of Flat Epithelial Atypia (FEA) on Mammotome Core Needle Biopsy: Should It Be Excised?

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Background: While a diagnosis of atypical ductal hyperplasia (ADH) at core needle biopsies (CNB) is an indication for surgical excision, the management and significance of flat epithelial atypia (FEA) is unclear. The aim of this study was to review CNB with atypia to determine incidence, types, associations and significance of FEA. We also investigated the utility of the proliferation marker Ki-67 in predicting which ADH or FEA were associated with carcinoma on excision.

Design: Consecutive CNBs performed between January 2000 and June 2005 with a diagnosis of atypia were reviewed by two pathologists. ADH and FEA were diagnosed following published criteria and categorized as pure FEA (no associated ADH), pure ADH (no associated FEA), or both. We recorded the following parameters: microcalcifications, inflammation, and myxoid change and fibrosis. Specific features of FEA such as a low-power pattern, the presence of apocrine cells, and/or cystically dilated ducts were recorded. Follow-up excisional biopsies were reviewed for all patients. Ki-67 immunostain was performed on all CNBs using the MIB-1 antibody, and interpreted as the percentage of epithelial cells with nuclear staining.

Results: Forty nine CNBs from 45 patients (age range 38-89 years) were studied. Seven of 49 CNBs (14.3%) had pure ADH, 9 of 49 (18.4%) had pure FEA, and 33 of 49 (67.3%) contained both ADH and FEA. The most common pattern of FEA was that of a well circumscribed group of ducts resembling blunt-duct adenosis (50%), followed by cystically dilated ducts with secretions (33%), and less commonly with apocrine features (7%). Chronic inflammation was seen in 47%, and fibrosis and myxoid change in 39% of FEA. Excisional biopsies performed in 39 of 46 patients showed that 9 patients (23%) had DCIS and/or invasive carcinoma, 4 (10%) had LCIS or ALH, 9 (23%) had residual ADH, and 17 (37%) had no atypia. Of the 9 CNBs with pure FEA, 3 (33%) were upgraded to DCIS and/or invasive carcinoma on excision. The percentage of FEA and/or ADH cells staining for Ki-67 was similar (mean 3%, range 0-10%) regardless of whether they were upgraded to carcinoma or not on excision.

Conclusions: There is a strong association between FEA and ADH which may reflect a biologic progression. Most FEA have a low power appearance of a well-circumscribed group of ducts, and is commonly associated with chronic inflammation and stromal changes. FEA shows a risk of upgrade to carcinoma similar to that of ADH and hence it should be recognized and warrants follow-up excision.

136 Clinicopathologic Parameters and Biological Markers Predicting Nonsentinel Node Metastasis in Sentinel Node Positive Breast Cancer Patients

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Background: Sentinel lymph node (SLN) biopsy is the standard procedure for axillary conservation in invasive breast cancer (IBC) patients without SLN metastases. However, the value of complete axillary node dissection (ALND) has been questioned in IBC patients with positive SLN because more than 50% of patients with SLN metastasis do not have nonsentinel lymph node (NSLN) involvement.

Design: To find out which patients are likely to have NSLN metastasis, 205 IBC patients with at least one positive SLN managed by SLN biopsy and ALND at National Cancer Center between Jan 2002 and Dec 2004 were included. We examined the clinicopathologic characteristics of the primary tumors, SLNs and NSLNs. We also evaluated the biological markers of the primary tumors with tissue microarray and immunohistochemistry.

Results: Eighty-nine patients (43.4%) had additional metastases in NSLNs of 205 patients with SLN metastases. The following factors were found to be associated with NSLN metastases; peritumoral lymphovascular invasion ($p = 0.01$), two or more metastatic SLNs ($p < 0.01$), SLN metastases > 2 mm ($p < 0.01$), and extranodal extension ($p < 0.01$). Primary tumor > 2 cm showed more NSLN metastases, but the association was statistically insignificant ($p = 0.08$). However, histologic grade, histologic type, presence of extensive intraductal component, presence of high grade ductal carcinoma in situ, and the number of harvested SLNs were not associated with NSLN metastases. E-cadherin, CD44, cyclin D1, p21, ER, PR, c-erbB2, p53, Ki-67, luminal (CK7, CK18, CK19) and basal (CK5, p63) markers were not useful predictors for NSLN metastasis in IBC patients with SLN metastases. Multivariate analysis revealed that SLN metastasis > 2 mm ($p = 0.01$), two or more metastatic SLNs ($p = 0.03$), and extranodal extension ($p < 0.01$) were independent predictors for NSLN metastases.

Conclusions: For the prediction of NSLN metastasis in IBC patients with SLN metastases, the light microscopic evaluation of the number, size, and extranodal extension of metastatic SLN by hematoxylin and eosin staining appeared to be critical practice, while the characteristics of primary tumor by immunohistochemical staining for luminal and basal markers, hormone receptors, E-cadherin, CD44, cyclin D1, p21, c-erbB2, p53, and Ki-67 did not appear to be helpful predictors.

137 Correction for Chromosome 17 Is Critical for Determination of True HER2 Gene Amplification Status in Breast Cancer (BC)

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Background: Accurate assessment of Her2 amplification is critical in BC management, as it is the best predictor of response to trastuzumab-based therapy. The aim was to determine the best scoring system for Her2 amplification status, particularly for BC with polysomy-17.

Design: Her2 and CEP17 copy number were evaluated by FISH on 893 BC samples. Three criteria of FISH amplification were used: ratio Her2/CEP17>=2, absolute number of Her2>4 or >6. HER-2 protein expression was evaluated by IHC for 584 samples and gene expression by mRNA assay NASBA for 82 frozen samples. Standard descriptive analyses were conducted. Association between parameters was assessed using concordance coefficients Kappa, Mann-Whitney and Chi-square tests.

Results: Samples were divided in 5 groups: amplified (A) and not-amplified (NA) disomic, A and NA polysomic and discordant polysomic representing 10.8% of cases (most of them being + with Her2>4 criteria and - with the 2 others). FISH criteria's best association was seen between ratio and Her2>6 (.906, $p<10^{-6}$). With IHC 3+ as positive, best associations were seen with Her2>6 (.689) and ratio (.650) criteria (all $p<10^{-6}$). Best association NASBA /FISH was seen with ratio criteria ($p<10^{-6}$). Polysomy had an impact on Her2 copy number: it was higher in poly-versus disomic BC (all p<.02) and CEP17 copy number was higher in A versus NA (all p<.03). Nevertheless, polysomy did not have an impact on Her-2 protein and mRNA as no difference was observed between poly- and disomic samples. Polysomy seemed to have an impact on proliferation: a lower % of Ki67>25 samples were found in the NA disomic group compared to the NA polysomic (p=.03), but the same was not true for the A groups. Discordant samples presented a highest CEP17 copy number compared to the other polysomic groups (all p<.003), although HER2 protein and mRNA levels were similar to those of the NA samples. Interestingly, discordant BC had a % of ER+ samples similar to the NA group, and statistically different from the A group (p=.004). Among discordant samples, a perfect concordance between FISH/IHC/NASBA (binary data) was observed in 66.7% cases with ratio criteria, in 60% with Her2>6 and in 0% with Her2>4.

Conclusions: Her2 gene copy induced by polysomy in BC were not associated with higher protein and mRNA levels. Best concordance between FISH/IHC/NASBA was observed with the ratio Her2/CEP17 criteria.

138 Analysis of Breast Cancer Risk in Women with Fibroadenoma

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Background: Fibroadenomas (FA) are common benign lesions comprised of stromal and epithelial elements. Studies have shown an increased risk of breast carcinoma development in women with FA, with relative risks varying from 1.6 to 3.88.

Design: Fibrocystic lesions were histopathologically classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH) in a benign breast disease cohort (BBD) of 9363 who underwent biopsy from 1967-1991. Fibroadenoma (FA) assessment was also performed. Simple FA contained no ductal hyperplasia (within the FA or in accompanying breast tissue), adenosis, or radial scar/papilloma. These were classified as NP lesions. FA associated with or containing the above proliferative lesions or AH were classified as PDWA or AH, respectively. The relative risk of breast cancer development within our cohort was compared to that expected in the general population using standardized incidence ratios (SIRs, mean follow-up interval 17 years).

Results: Of the 9363 BBD cases, 2219 (23.7%) contained FA, with subsequent carcinoma development in 167 (7.5%) of the FA cases. Ninety (5.5%) of the 1644 NP cases with FA subsequently developed carcinoma. Five hundred twenty-six patients were diagnosed with PDWA + FA, and 67 (12.7%) of these developed carcinoma. Forty-nine cases of AH + FA were identified, and 10 (20.4%) of these developed carcinoma. The relative risk of cancer development for FA versus non-FA groups is presented in Table 1.

Incident breast cancer relative risk

Diagnosis	Number Eligible Women	Relative Risk (95% CI)
NP	4591	1.14 (1.02, 1.27)
NP + FA	1644	1.16 (0.93, 1.43)
PDWA	2260	1.65 (1.44, 1.88)
PDWA + FA	526	2.37 (1.84, 3.01)
AH	293	3.94 (3.00, 5.07)
AH + FA	49	4.51 (2.16, 8.29)

Conclusions: Presence of simple FA imparts no increased risk of breast cancer development compared to NP changes. However, patients with FA and PDWA have a slightly greater risk than those with PDWA and no FA. The identification of AH in patients with FA does not appear to modify the cancer risk attributable to the former.

139 Neutrophil Gelatinase-Associated Lipocalin (NGAL) Is Highly Associated with HER2+/ER- Breast Cancers and Is a Downstream Effector of PI3/AKT Pathway

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Background: NGAL is a secretory protein involved in multiple biological functions. Previous breast cancer cell line experiments have shown that NGAL binds to matrix metalloproteinase-9 (MMP-9) and protects MMP-9 from degradation and thereby enhances its enzymatic activity and facilitates angiogenesis and tumor growth in breast cancer. However, NGAL expression has not been previously studied in primary breast cancers and its molecular pathway is not fully illustrated.

Design: NGAL expression and its associated genes were analyzed by gene expression profiling from 82 primary breast cancers. NGAL expression was further confirmed by immunohistochemical staining. MCF-7 human breast cancer cell lines were transfected with HER2/neu, Akt active and inactive genes to determine the molecular pathway of NGAL.

Results: Statistical analysis of gene profiling data from the 82 primary breast cancers showed that NGAL expression is associated with ER negative status ($P < 0.0001$), HER2/neu gene overexpression ($P = 0.003$) and combined HER2+/ER- ($P < 0.01$). Transient transfection of MCF-7 cells with active HER2/neu and Akt gene shows elevated NGAL expression compared to transfection of inactive/dominant negative mutants of HER2/neu and Akt.

Conclusions: NGAL is a secretory protein involved in tumor growth of breast cancers. This study is the first to illustrate that HER2/neu-Akt pathway is involved in NGAL tumor progression of breast cancers.

140 Number of Clusters of Isolated Tumor Cells in Sentinal Lymph Nodes Correlates with Tumor Grade but Not with the Number of Prior Procedures in Breast Carcinoma

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Background: The clinical significance of isolated tumor cells (ITC) in sentinel lymph nodes (SLN) of breast carcinoma patients is unknown. Currently it is not included in the staging procedure for breast cancer patients. It was suggested that breast epithelial cells may be transported to SLN by benign mechanic transport during surgery, mammography, or sentinel lymph node biopsy. We aim to study breast carcinoma patients with only isolated tumor cell deposits to determine if the number of ITC deposits is related to tumor characteristics or the number of prior procedures.

Design: 35 cases of breast carcinoma with ITC deposits were retrieved from the surgical pathology files of authors' institution. ITC was identified by immunohistochemical stain for epithelial marker pancytokeratin with each cluster measuring < 0.2 mm. No patient had a sentinel lymph node involvement by a micro- or macro-metastatic carcinoma. The number of ITC clusters were counted and its relationship to patients' age, primary tumor size, modified Bloom-Richardson grade, number of prior procedures including fine needle aspiration, needle core biopsy and lumpectomy were investigated.

Results: The patients' median age was 53.5 (range from 34 to 80). On average 3.6 (range 1 to 8) sentinel lymph nodes were sampled in these patients. An average of 3.7 (range 1 to 22) clusters of ITC were identified in a mean of 1.4 (range 1 to 5) sentinel nodes. The number of ITC did not correlate to the number of prior breast procedures (P=0.57). It also did not correlate with patients' age, the size of primary tumor, or type of breast caner (invasive ductal vs lobular carcinoma). However, the number of ITC correlated with modified Bloom-Richardson grade, with more ITC seen in breast carcinoma with higher grade (P=0.007).

Conclusions: Our study shows that the number of ITC did not correlate with the number of prior surgical procedures, suggesting that ITC could not be entirely explained by the mechanical displacement during manipulations of breast tissue. Rather, it correlated with higher tumor grade, indicating the clinical significance of ITC needs further investigation.

141 Histopathologic Predictors of Malignancy in Breast Core Needle Biopsy for Papillary Breast Lesion

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Background: Papillary lesions (PL) of the breast are characterized by an intraductal epithelium proliferation with papillary arborescent growth pattern supported by fibrovascular cores with or without myoepithelial cells. PLs of the breast are commonly encountered in routine surgical pathology and consist of a heterogeneous group that includes papilloma, atypical papilloma, carcinoma arising in a papilloma and invasive papillary carcinoma. In current surgical pathology practice, there are several problems regarding diagnosis of papillary lesions encountered in the breast needle core biopsy (CNB) specimens. As a result most of the practicing pathologists will recommend surgical excision following such a diagnosis.

Design: The purpose of this study is to determine the accuracy of CNB diagnosis of papillary lesions of breast and to identify histological features that may predict malignant changes. Retrospectively, two surgical pathologists reevaluated the histopathologic features of twenty-nine cases of CNB with a previous diagnosis of papillary lesion and correlated these with the radiology interpretations and the excision findings in a blinded manner.

Results: Seventeen CNB cases (17/29) are identified as papillomas (PL_1), 3 cases (3/29) as atypical papillomas (PL_2), and 4 cases (4/29) as malignant papillary lesion (PL_3). The findings in CNB of these 24 cases were concordant with the findings in the excision specimens. Five cases however, show discordant results when compared with the excision findings. Four cases were diagnosed as PL_2 on CNB show carcinoma arising in papilloma and one case of PL_1 on CNB shows atypical papilloma on excision.

Conclusions: CNB specimens are valuable material when breast image reports are taken into account during histologic evaluation of papillary lesions. Monotonous complex epithelial cells hyperplasia, cellular atypia, or lack of myoepithelial cells in papillary lesions are accurate predictions of malignancy in papillary lesions and the grounds for recommendation of surgical excision.

142 Identification of a Basal-Like Subtype of Ductal Carcinoma In Situ

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Background: Microarray profiling of invasive breast carcinomas has identified distinct subtypes of tumors (luminal A, luminal B, HER2 overexpressing, and basal-like) associated with different clinical outcomes. The basal-like subtype is associated with poor clinical outcomes and is the subtype observed in BRCA1-related breast cancers. Invasive basal-like tumors have been immunohistochemically characterized as being ER negative, HER2 negative, and positive for either cytokeratin 5/6 or EGFR. It is presumed that a basal-like in situ counterpart exists for these tumors. The aim of this study was to evaluate for the presence of an in situ basal-like subtype.

Design: A total of 275 pure DCIS cases from the Carolina Breast Cancer Study, a large population-based case-control study group, were evaluated for histologic characteristics and then immunostained for ER, HER2, EGFR, cytokeratin 5/6, p53 and ki-67. Cases were categorized as luminal A (ER+, HER2-), luminal B (ER+, HER2+), HER2 positive (ER-, HER2+) or basal-like (ER-, HER2-, EGFR or CK 5/6+) based on immunophenotype.

Results: Each subtype was identified within the in situ breast carcinomas including 20 (7%) basal-like, 171 (62%) luminal A, 25 (9%) luminal B, and 43 (16%) HER2+/ER-. Sixteen (6%) tumors were unclassified, negative for all 4 defining markers. The prevalence of the basal-like subtype was 6% in the African-American population, 8% in the non-African-American population, and 7% in both premenopausal and postmenopausal women. Among the various DCIS lesions, the basal-like subtype showed a higher frequency of comedo-type histology (68%, p<0.0001), p53 positivity (63%, p<0.0001), and high (>25%) ki-67 index (33%, p<0.0001).

Conclusions: A basal-like subtype of ductal carcinoma in situ was identified in our Carolina population group with a frequency of approximately 7%. A statistically significant association between race or menopausal status and the basal-like subtype was not identified. The basal-like DCIS cases showed a higher frequency of comedo-type histology, p53 positivity and high ki-67 index. The frequency of the basal-like subtype in DCIS lesions appears to be less than the estimated 15% frequency reported for invasive carcinomas. The relative infrequency seen in the pure in situ carcinomas may be secondary to the rapid progression of these tumors to an invasive malignant phenotype.

143 Detection of Occult Metastatic Carcinoma in Patients Who Had Initial Cytologically Proven Positive Axillary Lymph Nodes with Subsequent Complete Pathologic Response Following Pre-Operative Chemotherapy for Locally Advanced Breast Cancer. An Immunohistochemical Study

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Background: Preoperative chemotherapy (PCT) is considered the standard of care for the management of locally advanced breast cancer and is increasingly being used for earlier stage breast cancer. Complete pathologic response (eradication of both primary tumor and lymph node (LN) metastases) predicts an excellent probability of long-term survival. Although clinical significance of occult nodal metastasis in patients (pts) with breast cancer is extensively studied in general, their prognostic value in pts with locally advanced breast cancer after PCT is not known. In this study we evaluated the detection rate and clinical significance of occult nodal metastases in LNs that contained metastatic carcinoma at the time of initial diagnosis and converted to negative following PCT.

Design: 44 pts with cytologically positive LNs that converted to negative following PCT were identified from two prospective clinical trials. All LN sections were reviewed, one deeper level H&E section was obtained and immunohistochemical staining for cytokeratin (CK) was performed. A total of 762 LNs were evaluated for occult metastases. Histologic features of therapy related changes were assessed. Kaplan Meier survival curves were evaluated for calculating the relapse free and overall survival.

Results: Occult metastases were identified in 8/44 (18%) pts. In six pts there was only 1 node with occult metastasis, in 1 pt, 2 nodes and in 1 pt, 3 nodes. In 7 pts only isolated CK positive cells were identified. In one patient the positive LN had tumor cell clusters measuring 0.35 mm, (evident on recut H&E and CK). In all cases occult metastatic carcinoma cells were embedded within areas of fibrosis, foreign body giant cell reaction and extensive histiocytosis. Pts with occult metastases had more frequent residual primary breast cancer than node negative pts; 4/8 pts with occult metastases had residual primary disease compared to 7/36 LN negative pts. There was no statistical difference between either recurrence free or overall survival rates of pts with or without occult metastases (median follow-up 61 months).

Conclusions: Our data shows that occult metastases in pts with locally advanced breast cancer after PCT is not a rare event. However these occult metastases do not have prognostic significance. Therefore, routine CK evaluation of these LNs is not recommended.

144 Predictive Biomarkers of Breast Cancer Response to Neoadjuvant Chemotherapy and Their Relationship to Pathologic Assessment of Residual Disease

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Background: Pathologic complete response (pCR) following neoadjuvant chemotherapy is associated with improved survival but is less common in estrogen

receptor (ER) positive breast cancer. We evaluated the association of ER and other biomarkers with the extent of pathologic residual disease (pRD).
Design: A tissue microarray was constructed from 144 breast cancer samples acquired before neoadjuvant chemotherapy [paclitaxel, then 5-fluorouracil, doxorubicin, and cyclophosphamide (T/FAC)], and stained for ER, PR, HER2, bcl-2, beclin1 and tau. Tumor response was defined : 1. pCR versus pRD, 2. residual tumor size (pT0, ≤ 1cm, > 1cm), and 3.a new parameter to evaluate residual invasive cancer burden (RCB) within the breast and axillary nodes.
Results: pCR was achieved in 26 % of patients, but was significantly less frequent in tumors that expressed ER, tau , PR , bcl-2, or beclin 1. There was significantly more RCB in tumors that expressed ER (p<0.001), bcl-2 (p=0.001), beclin 1 (p=0.003), or tau (p=0.02), but not PR.Breast cancers that coexpressed ER, bcl-2 and tau (23%) frequently had pRD (93%), were >pT1c (81%), and more extensive RCB(Table 1).
Conclusions: Expression of ER or tau was associated with pRD. Expression of ER, beclin 1 or bcl-2 was associated with more extensive RCB. The subset of breast tumors that coexpressed ER, bcl-2 and tau had the most extensive residual disease after neoadjuvant T/FAC chemotherapy.

ER, bcl-2 and Tau expression versus: pCR vs. pRD, residual pT stage and RCB (N=120)

Parameters of response		ER negative	ER positive	ER, Bcl-2 positive	ER, tau positive	All positive
pCR vs. pRD	pCR	49%	18%	25%	75%	7%
	pRD	51%	82%	75%	84%	93%
Residual pT stage	pT 0	49%	18%	33%	21%	7%
	pT1a,b	27%	24%	8%	32%	11%
	≥; pT1c	24%	59%	58%	47%	81%
RCB (log scale) Mean rank		44.0	70.8	67.3	59.3	79.3

145 Follow-Up of Flat DIN 1 on Core Needle Biopsy

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Background: The clinical significance and optimal management of flat DIN1 (flat epithelial atypia) detected on core needle biopsy (CNB) are unknown. Although there is increased frequency of flat DIN1 on CNB, uniform management guidelines are lacking.
Design: The files of the Yale University Department of Pathology were searched for breast CNB evaluated between Jan 1992 and Dec 1999. CNB showing other than invasive malignancy, DCIS, LIN and fibroadenomas were retrieved. Cases with pure flat DIN1 as the most advanced lesion on CNB and no clinical evidence or history of in situ or invasive carcinoma in the same breast were included. Subsequent biopsies were retrieved and reviewed. Follow up was obtained from the medical records.
Results: Among 736 CNB reviewed, 63 qualified as flat DIN1 and met the criteria for inclusion. The median patient age was 52 years (range 39-75 years). The mammographic abnormalities leading to CNB were calcifications in 48 cases (76%) and density in 14 cases (24%). Follow up was available in 55 cases. Mean follow up was 6.2 years (range 1-11 years). Of the 63 patients, 24 had a subsequent breast biopsy (core or excisional) within 15 days to 10 years (16 in the ipsilateral and 4 in the contralateral breast, and 4 in both ipsi and contralateral breasts). An infiltrating carcinoma was found in 9 (13.8%) patients in a subsequent biopsy, 7(77%) in the ipsilateral breast and 2(23%) in the contralateral breast. The mean interval between the initial CNB and the invasive cacinoma was 3.7 years (range 2-8 years) for the ipsilateral tumors and both cases with contralateral tumor were found 7 years after the initial CNB. Five of the 24 patients underwent an excisional biopsy <3 months from the initial CNB, in none of these patients an invasive or intraepithelial carcinoma was identified in the excision.
Conclusions: Flat DIN 1 is a marker of increased risk for subsequent development of invasive breast carcinoma. When pure flat DIN 1 is found on CNB, additional levels (beyond the usual 3) should be performed to exclude a more advanced lesion. If arcades or micropapillae become apparent, the lesion should be diagnosed as AIDH, and an excisional biopsy should be performed. After correlation with mammographic findings and if additional levels fail to reveal a more advanced lesion, an excisional biopsy ismandatory; close follow up is advised with repeat mammogram every 6 months for 2-3 years for early detection of clinically occult invasive carcinoma in the vicinity of flat DIN1.

146 Comparison of Pathologic Features of Screen Detected (SDC) and Interval (IC) Breast Cancers in African American (AAW) and White (WW) Women

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Background: AAW with breast cancer historically have had poorer prognosis than WW. Data from the Carolina Mammography Registry (CMR) were reviewed to determine the pathologic characteristics of breast cancers from AAW and WW for both SDC and IC to determine if significant differences exist between these groups of women.
Design: CMR is a population-based registry that prospectively collects data from mammography facilities from 39 counties in NC. Corresponding pathology data is collected from hospitals and laboratories serving this population and from the state Central Cancer Registry. For this study, pathology data (cancer type, size, grade, stage and receptor status) from SDC (those detected by a screening mammogram within 1 year) and IC (those that present between screening intervals) for AAW and WW from 1994-2002 were compared using Cochran-Mantel-Haenszel Chi-square statistics.
Results: There were 3900 cancers among the 311,430 women in the CMR (SDC: 510 in AAW, 2385 in WW; IC: 173 in AAW, 832 in WW). Significant diffrences in the distributions of characteristics were as follows: For tumor stage, SDC were more likely to be in situ than IC in WW(15.1 vs. 9.5%, p<.001). Cancer type (ductal vs. lobular) was more likely to be lobular in WW than AAW for IC, and in WW was more likely to be lobular in IC than SDC (9.6% vs. 5.6%, p<.001). AAW were more likely to have larger tumors than WW in SDC, in both AAW and WW, IC were significantly larger than SDC (p<.001), and there were no significant racial differences in tumor size in IC. Tumors were more likely to be poorly differentiated in AAW than WW in both SDC and IC, and IC were more likely to be poorly differentiated than SDC for both AAW and WW (p<.001 for all). WW were more likely to have estrogen and progesterone receptor positive tumors than AAW (72 vs 55%, p<.001), with minimally significant differences between ER and PR positivity for SDC vs IC in AAW and no significant differences for SDC vs IC in WW.
Conclusions: Significant differences were found between SDC and IC and between races. As has been reported previously, IC were more likely to be invasive carcinomas, larger tumors, lobular type and poorly differentiated than SDC. AAW were more likely to have larger tumors, poorly differentiated tumors and hormone receptor negative tumors than WW. The increase in proportion of invasive tumors, increase in tumor size and increase in lobular type in IC was predominately driven by racial differences in this study.

147 Cytologic Features of Breast Cancers with a Basal Phenotype

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Background: Recently the aggressive potential of breast cancers with a basal-like phenotype (ER,PR, Her-2 negative, cytokeratin 5 positive) has become apparent. However, their cytologic features have not yet been described. We studied the frequency of these tumors in our patient population in correlation with the age, race and tumor size at presentation and their cytologic features.
Design: 97 consecutive cases of breast cancer diagnosed on FNA from 2003 to 2005 which had marker studies performed on the cellblock were reviewed. Patient data included age, race and tumor size at presentation, ER, PR and Her-2 expression.
Results: The age range of our patients was 26-96, of which 28 were less and 69 more than 45-years old. and 89% were Afro-American.

Distribution of breast cancer marker studies in correlation with the age

Markers/Age	I: ER, PR, Her-2 positive	II: ER, PR positive, Her-2 negative	III: ER, PR negative, Her-2 positive	IV: ER, PR, Her-2 negative	Total *
Less or 45 year-old	0	10 (36%)	1 (4%)	13 (46%)	28 (29%)
More than 45 year-old	8 (12%)	29 (42%)	4 (6%)	19 (28%)	69 (71%)

Her-2 2+ cases not confirmed by FISHare not included in the table

In 59 (61%) of cases the marker studies performed on excisional specimen showed similar results. The average tumor size at presentation was 3 to 4 cm in the first three groups in both ages, and 6 cm in the triple negative group. Cytologically, the triple negative tumors (showing a basal phenotype) were highly cellular with high dregree of dyshesion, pleomorphic nuclei and frequent nuclear pyknosis or karyorrhexis.
Conclusions: Tumors with triple negative markers were equally distributed in women younger and older then 45 and tended to be larger. Well performed FNA of breast cancer obtains material for an accurate morphologic diagnosis and allows performance of marker studies, allowing accurate classification of breast cancers into the recently described, prognostically significant tumors of luminal and basal-like phenotypes. Cytologic features are characteristics, but not pathognomonic for tumors with a basal-like phenotype. Applications of additional stains, including p63, cytokeratin 5 and Ki-67 to tumors with triple negative markers is necessary to accurately classify these tumors.

148 Phenotypic Prognosis of Node-Negative Breast Cancer in the Genotypic Era?

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Background: Recent reports that gene-specific RNA assays can predict node-negative breast cancer outcome with clinically useful accuracy have stirred much interest (Paik S *et al*, N Engl J Med 2004;351:2817). We will show similar results by use of phenotypic markers.
Design: The population studied comprises 631 patients treated definitively at St. Luke's Hospital from 1985-1997. Four independently prognostic variables: size (S), lymphatic invasion (I), tubule-formation (D), and DNA precursor uptake index by *in vitro* labeling (LI) were defined (Meyer JS *et al*, Mod Pathol 2005;18:1067). This report addresses how best to combine these variables to define a group of patients with prognosis sufficiently good to influence therapy. Median duration of surveillance after diagnosis was 6.4 years. In-breast relapses were not classified as events.
Results: The table shows disease-specific relapse-free survival (RFS) rates for groups with various combinations of favorable prognostic markers. The last three rows show the maximum number of patients that could be assigned a good prognosis (N=238, RFS >95%), together with a group with poor prognosis (N=373), and a small third group (N=20). The group with good prognosis comprises 38% of patients. This result compares favorably with the low risk genotypic group of Paik *et al* (51% with distant relapse-free survival of 93.2%). Chemotherapy might be withheld from these patients. LI >8% defined a group (32%) with high risk but no relapse after 6 years (cured fraction = 80%).
Conclusions: Phenotypic assay utilizing four independently prognostic variables comprising pathologic tumor size, presence or absence of lymphatic invasion, degree of tubular formation and a reproducible proliferation measurement has potential for clinically meaningful breast cancer prognosis. In efficacy it is competitive with gene product assay. With measurement of proliferation by Ki-67 immunoassay quantified by image analysis it could be applicable in many pathology laboratories.

Combinations of Risk Category Specifications, Number of Patients Selected, and 8 Years RFS

S, cm	I (0-2+)	D (0-2+)	LI (%)	Number (%)	RFS	95% CL
≤1	Any	Any	Any	165 (26)	.961	.93-.99
Any	Any	Any	≤3	253 (40)	.946	.91-.98
Any	Any	Any	≤2	179 (28)	.959	.92-.99
≤2	0	1-2+	≤3	129 (20)	.963	.93-1
≤2[1]	'	'	'	238 (38)	.957	.93-.99
>2	0	1-2+	≤3	20 (3.2)	.941	.83-1
>1[2]	Any	Any	Any	373 (59)	.824	.78-.87

1. Includes all tumors ≤1 cm with I=0, and 1.1-2 cm tumors with I=0, D=1-2+, LI≤3. 2. All patients not otherwise classified.

149 Abstract Withdrawn

150 The Impact of Lobular Involution on Breast Cancer Risk

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Background: Lobular involution is a histologic finding that reflects atrophy associated with physiologic aging in the human breast. Based on epidemiologic associations, involution has been hypothesized to have relevance in breast tumorigenesis.

Design: A breast pathologist examined benign breast biopsies of 8,743 women in the Mayo Benign Breast Disease cohort and classified them according to the degree of lobular involution as follows: none (0%), partial (1-74%), or complete (>75%). Each benign biopsy was also evaluated per standard criteria as nonproliferative (NP), proliferative disease without atypia (PDWA), and atypical hyperplasia (AH). Age at biopsy, family history of breast cancer, and development of breast cancer were obtained from medical records or questionnaires (17-year mean follow-up). Associations of involution with other breast cancer risk factors were carried out using chi-square tests and logistic regression analyses. Relative risks of breast cancer were estimated by comparing the number of observed events with the number expected based on rates from the Iowa SEER registry.

Results: Distribution of the patients by the three levels of involution was as follows: none-1,628 (18.6%); partial-5,202 (59.5%); and complete-1,913 (21.9%). Increased involution was found to correlate with increased age and decreased family history of breast cancer. The relative risk of breast cancer was significantly lower in patients who had complete (0.91, 95% CI 0.74-1.10) compared to those with partial (1.45, 95% CI 1.32-1.59) or no involution (1.88, 95% CI 1.59-2.21) ($p<0.001$). Age and family history modified breast cancer risk. In patients with PDWA, the relative risk for women with no involution was (2.94, 95% CI 2.26-3.75), while that for women with complete involution was only (1.11, 95% CI 0.68-1.72) ($p<0.001$). The relative risks in patients with NP and AH displayed similar associations.

Conclusions: The degree of lobular involution correlates inversely with breast cancer risk. It modifies breast cancer risk in patients stratified by age, family history, and type of histology. These data indicate that aberrant or delayed involution is a biologically important constitutional variable in breast cancer biology.

151 Extensive Sampling of the Surgical Specimens Is Essential for Accurate T-Staging in Patients with Infiltrating Lobular Carcinoma of Breast: A Comparative Study of Gross Versus Microscopic Tumor Sizes

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Background: A clear inverse relationship between the size of the tumor and ten year survival has been established. Measurement of the greatest diameter of tumors in the surgical specimens is required for an accurate T-staging. According to the American Joint Committee on Cancer, tumors with dimensions of ≤2 cm, >2 - ≤5 cm, and >5 cm are staged as T1, T2, and T3. Infiltrating lobular carcinoma (ILC) of breast represents 7-10% of all invasive breast cancers. The borders of this tumor are difficult to delineate grossly. Since the size of the tumor is a major factor in staging of the lesion, we propose extensive sampling of the specimen and microscopic resizing are needed to T-stage the ILC tumors accurately. The sampling would include part of the specimen that did not contain grossly visible tumor.

Design: A retrospective study was performed on the specimens of 76 cases with ILC at the UCLA Medical Center between 2003 through 2005. The patients' ages ranged from 38 to 95 years. Specimens were from lumpectomy and mastectomy on 36 and 40 patients respectively. The specimens had been extensively sampled and mapped. Resizing of tumors was performed by marking the microscopic extensions and compiling the measurements. The cases were grouped into T1, T2, and T3 according to the recorded gross sizes. Then, microscopic restaging was carried out within each of the initial three groups.

Results: The results of the T-staging are listed in the Table. In Group1, 52 cases were staged as T1 grossly. When microscopically restaged, 40% of them changed to T2 (27%) and T3 (13%). In Group 2, 47% restaged as T3 microscopically. All 7 cases (100%) in Group 3 remained as T3.

Conclusions: Based on this study, it becomes clear that the gross T-staging of the ILC tumor may not be accurate when the size of the lesion is 5 cm or less. These results show that the gross measurements alone may underestimate 40-50% of the tumor T-stages. Therefore, if the gross size of the tumor is 5 cm or less, an extensive sampling of the specimen is needed for accurate T-staging of the tumor following microscopic size determination.

	Group 1, Gross T1		Group 2, Gross T2		Group 3, Gross T3	
Gross Sizes	Gross, n	Microscopic, n (%)	Gross, n	Microscopic, n (%)	Gross, n	Microscopic, n (%)
0 - ≤;2 cm (T1)	52	31 (60%)		0 (0%)		0 (0%)
>2 - ≤;5 cm (T2)		14 (27%)	17	9 (53%)		0 (0%)
>5 cm (T3)		7 (13%)		8 (47%)	7	7 (100%)

152 The Challenge of the Dominantly In Situ Breast Carcinoma to Stage and Grade

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Background: As the average size of breast cancers has decreased, the precision of measurement of smaller cancers is a challenge. Ascertainment has been aided by the mandate that size of the invasive portion be verified microscopically. Separate identification and recording of the extent of in situ disease is also required. We have assembled a group of patients whose breast cancers characterize a special and little discussed group that have: mass lesions with relatively clear circumscription; dominant central pattern of ductal carcinoma in situ (DCIS); and peripheral foci of apparent invasion, each clearly 3-5 mm or less, sometimes merging with the central mass but having an infiltrative growth, and lacking myoepithelial (ME) markers peripherally. This is important because such lesions are often mis-characterized as larger lesions.

Design: Twenty one cases seen in consultation at Vanderbilt University with well circumscribed central lesions with small foci of peripheral invasion measuring <5 mm were reviewed. Immunohistochemical stains for ME markers including smooth muscle actin and p63 were performed on a subset of these lesions. A diagnosis of microinvasion or minimal invasion was defined as extension of the tumor beyond the in situ component and specialized connective tissue, demonstrating an invasive appearance, and loss of the ME markers.

Results: All patients were woman who ranged in age from 32-79 years, with a mean age of 54 years. The mean size of the overall tumor including the invasive and in situ components was 1.6 cm (range 1.0 cm-4.2 cm). The mean size of the invasive component was 2.3 mm (range <1.0 mm-5 mm). All lesions showed a central focus of DCIS with predominantly cribriform and solid patterns. The invasive component was predominantly a solid variant of invasive cribriform carcinoma (54%) with the remaining tumors being invasive mammary carcinoma, no special type (46%). In the subset of cases (n=8) with immunohistochemistry for ME markers, the small foci with an invasive pattern showed total loss of the ME layer.

Conclusions: Although the American Joint Committee on Cancer staging protocol explicitly defines staging be based on the size of the invasive component, this is sometimes difficult to determine with precision. We present 21 large circumscribed lesions (overall size ≥ 1 cm) characterized by central DCIS and small foci of peripheral invasion measuring <1.0-5.0 mm, all of low and/or intermediate grade in both components. It is important to recognize such lesions to prevent overstaging and accurately classify these as T1a lesions.

153 ER Phenotype in Breast Cancer Remains Stable in Recurrence and Metastasis

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Background: The question of whether locoregional recurrence of breast cancer or distant metastases represent selective clones of tumor cells with phenotypes that render growth advantage has not been adequately answered. Specifically, with regard to the estrogen receptor, it is not clear if the ER phenotype of recurrent or metastatic breast cancer changes with or without treatment. In this study, we evaluated the ER phenotype of recurrent or metastatic breast cancers and compared it to the ER status of the primary tumor.

Design: Two hundred and thirty-six (236) patients with breast cancer who had synchronous metastasis (172) or metachronous local recurrence and/or metastasis (64) were included in this study. All primary, recurrent and metastatic tumors were evaluated for the immunohistochemical expression of ER using monoclonal antibody 1D5, heat-induced antigen retrieval and the L-SAB detection system (All from DakoCytomation, Carpinteria, CA).

Results: All patients had lumpectomy or partial mastectomy followed by other treatment modalities that included radiation and/or chemo- or hormonal therapy. One hundred seventy-two patients had metastatic carcinoma either in the axillary lymph nodes or in other sites at the time of diagnosis. The remaining 64 patients had local recurrence or distant metastasis that occurred from five months to 21 years after the diagnosis of the primary tumor. Overall 137 (58%) of primary tumors were positive for ER. In 228 patients (96.6%), the ER status of the primary and metastatic tumors were the same; i.e. either diffusely positive or completely negative. In eight patients with ER-positive primary tumors, the metastases (7 in axillary lymph nodes and one in bone) were ER-negative (3.4%). There were no patients with ER-negative primary tumor and ER-positive metastasis. Most of the 27 late-recurring or metastatic tumors were ER-positive; 92% after 10 years and 78% after five years.

Conclusions: When monoclonal ER antibody 1D5 is used, with rare exceptions, the ER phenotype of the primary breast cancer remains stable in recurrent or metastatic tumors. This is independent of the type of therapy. Therefore, if the ER status of the primary tumor is known repeat testing of the recurrent or metastatic tumors is unnecessary. Those breast cancers with late recurrence or metastasis are more likely to be ER-positive.

154 Over-Expression of the N-myc Downstrean-Regulated Gene 1 (NDRG1) Is Associated with Poorer Prognosis in Breast Cancer

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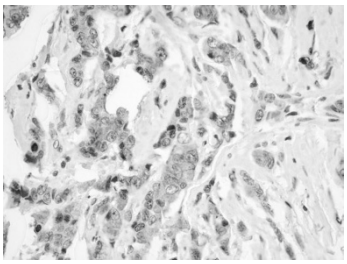
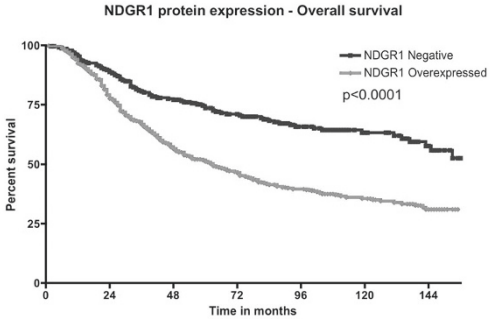
Background: The N-myc downstream-regulated gene 1 (NDRG1) encodes for a 43 kDa protein The function of NDRG1 is still unknown, however its expression was shown to be associated with cell proliferation, differentiation, apoptosis, tumorigenesis and metastasis. Few studies with a limited number of cases have been reported regarding NDRG1 expression in breast cancer, but clinical significance remain to be established. The aim of this study was evaluated NDRG1 expression and clinicopathological data in 720 breast carcinoma patients.

Design: Tissue samples were obtained from 720 breast cancer patients (median age=54yo). NDRG1 mRNA expression was determined in 74 tumors using quantitative Real Time PCR. NDRG1 protein expression was evaluated by IHC using TMA containing 699 primary invasive breast tumors. The histological grades (n=686) were G1 (n=144), G2 (n=386), and G3 (n=156), and 662 cases were classified as ductal carcinoma NOS. Lymph node metastasis were present in 308/696 cases.
Results: NDRG1 mRNA expression were classified as normal expression (51.4% of the cases), under-expression (16.2%), and over-expression (32.4%). Sixty-two percent (414/699) of the cases analyzed showed NDRG1 protein expression. Significant associations were found between NDRG1 protein expression and lymph node metastasis (p=0.02), advanced clinical stage (p=0.0001), lower nuclear grade (p=0.0001), presence of estrogen receptor (p=0.0001) and ERBB2 over-expression (p=0.0001). In addition, both NDRG1 mRNA and protein expression were directly associated with shorter disease free and overall survival of the patients.
Conclusions: Our results provide strong evidence that increased NDRG1 expression could play an important role in breast cancer behaviour and could serve as useful prognostic marker of disease outcome.





155 Immunohistochemical Stain (AE1/AE3) on Negative Sentinel Lymph Nodes: Is It Worth the Trouble?

AL Namiq, F Fan, O Tawfik, PA Thomas. University of Kansas Medical Center, Kansas City, KS.

Background: The importance of sentinel lymph node biopsies in nodal staging of patients with breast cancer has been demonstrated from previous investigations. A significant advantage of sentinel lymph node (SLN) mapping compared with conventional axillary dissection is identifying the lymph node(s) with the highest probability of harboring any metastases and reduced postoperative morbidity. Different protocols have been proposed combining multiple sections and cytokeratin immunohistochemical stain to examine negative SLN specimens. The aim of our study is to evaluate whether we should routinely perform AE1/AE3 immunohistochemical stain on negative SLN to detect micrometastases (MM) (<2.0 mm).
Design: A total of 225 frozen sections on SLN specimens from 64 patients with breast cancer accessioned between Aug 1, 2003 and July 31, 2005 were retrieved. Fifteen SLN were called positive at the time of the frozen sections and remained positive on the permanent sections. All 210 "negative" SLN specimens diagnosed by frozen section were reviewed. Multistep sections through the SLN block with three sections for hematoxylin-eosin (HE) and one additional for pankeratin (AE1/AE3) immunostaining were routinely performed.
Results: Of the 210 SLN specimens that were called negative on the frozen sections, 208 (99%) remained negative on multistep sections and AE1/AE3 immunostaining, 1 (0.5%) SLN revealed MM (<1.0 mm) on multistep section and confirmed by immunostaining, and 1 (0.5%) SLN revealed MM (<1.0 mm) on immunostaining AE1/AE3.
Conclusions: Our study indicates that AE1/AE3 immunostain did not detect macrometastases (>2.0 mm). It is time consuming, cost-ineffective, and of questionable prognostic significance.

156 Progesterone Receptor Immunohistochemistry of Antibody Clones PRA, PRB and PRAB: Correlation with Tumor Characteristics and Outcome in Breast Cancer

A Nassar, SM Waldrop, C Cohen. Emory University Hospital, Atlanta, GA.

Background: Estrogen (ER) and progesterone (PR) receptor status by immunohistochemistry (IHC) represents the standard of care in the treatment of patients with breast cancer. This study assesses the sensitivity, specificity, and predictive values of different clones of progesterone receptor antibodies, compared to in-house PR antibody (PR2). Results are correlated with tumor characteristics and outcome.
Design: Using tissue microarray (TMA), 93 breast cancers were studied. Clinicopathologic data and follow-up were obtained. IHC was performed after antigen retrieval using the Bond-max autostainer (vision Biosystems): PR2 (TMA) (M; clone PgR 636; 1:400; Dakocytomation); PRA (M; clone 16; 1:1500; Novocastra); PRB (M; clone SAN27; 1:1500; Novocastra) and PRAB (M; clone 16 and SAN27; 1:1500; Novocastra). Statistical analysis assessed sensitivity, specificity, and predictive values of each clone, using PR2 as the gold standard. Correlation with clinicopathologic factors and outcome was performed.
Results:

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive (FP) and false negative (FN) rates of the different clones of progesterone antibody

	PRA	PRB	PRAB
Sensitivity	100%	100%	89%
Specificity	15%	69%	77%
PPV	73%	89%	89%
NPV	100%	100%	77%
FP rate	27%	11%	11%
FN rate	0%	0%	23%

Correlation of antibody clones PR2, PRA, PRB, PRAB with clinicopathologic parameters and outcome

	PR2	PRA	PRB	PRAB
Age	>0.05	>0.05	>0.05	>0.05
Tumor Size	>0.05	>0.05	>0.05	>0.05
Histologic type	>0.05	>0.05	>0.05	>0.05
Tumor grade	>0.05	>0.05	>0.05	>0.05
Stage	>0.05	>0.05	>0.05	0.061
ER	>0.05	>0.05	>0.05	>0.05
Her-2-neu	>0.05	0.095	0.051	>0.05
Outcome	>0.05	>0.05	>0.05	0.052
Survival (overall and disease free)	>0.05	>0.05	>0.05	>0.05

p-value > 0.05 not significant

Conclusions: PRA and PRB are both sensitive but less specific with no false negative rate; whereas PRAB is less sensitive but more specific. PRA and PRB tend to correlate with Her-2 neu. PRAB tends to correlate with stage and outcome. None of the PR antibody clones correlate with overall or disease-free survival.

157 Significance of Intramammary Lymph Nodes in the Staging of Breast Cancer: Correlation with Tumor Characteristics and Outcome

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Background: Intramammary lymph nodes (IntraMLNs) have received little attention as potential prognostic indicators for patients with breast carcinoma and are largely overlooked by radiologists, pathologists and surgeons. The prevalence of intraMLNs has been reported to range between 1% and 28%. Histopathologic studies of breast specimens containing intraMLNs have revealed that these lymph nodes may be present in any quadrant of the breast and can yield a variety of pathologic findings, including metastatic carcinoma in the ipsilateral breast. Patients with intraMLN metastases who otherwise have stage I breast carcinoma have been reported to have a poorer prognosis compared with patients who have stage I disease in the absence of intraMLN metastases. However, the presence of intraMLN metastases does not appear to influence the survival of patients who otherwise have stage II breast carcinoma. In the current retrospective analysis, we assessed the clinical significance of intraMLNs and evaluated their role in predicting outcome in patients with breast carcinoma.
Design: Between 1995 and 2005, 116 intraMLN specimens were identified. 59 (50.8%), found in association with benign breast conditions, were excluded from the analysis. Of the remaining 57 (49.2%) patients whose intraMLN specimens were associated with primary breast carcinoma, primary tumor characteristics and axillary lymph node status were recorded. Outcome data were documented. Statistical analysis was performed to detect correlation between intraMLN and tumor characteristics as well as outcome.
Results:

Correlation of tumor characteristics, lymph node status and stage with intramammary lymph node metastases (n = 57)

Variable	p-Value
Tumor size status	0.035
Tumor grade	0.037
Lymphovascular invasion	0.1524
Axillary lymph node status	0.0001
Stage	0.0004

Correlation of intramammary lymph node metastases and outcome

	IntraMLN (+)	IntraMLN (-)	p-Value
3-year survival rate	94%	40%	
Overall survival			0.0006
Disease-free survival			0.0004

Conclusions: Predictors of intraMLN metastases include: tumor size, tumor grade, axillary lymph node status and tumor stage. Patients with intraMLN positive for metastases have a poorer 3-year survival rate, overall and disease-free survival than patients with negative intraMLN.

158 Estrogen Receptor Expression in Ductal Carcinoma In Situ (DCIS): The Effect of Patterns, Grade, Apocrine Differentiation and Heterogeneity

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Background: Adjuvant hormone therapy for DCIS is currently based on estrogen receptor (ER) and progesterone receptor (PR) expression. ER and PR are known to be more often negative in high grade DCIS. The influence of heterogeneity in patterns, grade and apocrine differentiation on ER/PR expression in DCIS are not well known.
Design: ER & PR immunostains of 104 cases of DCIS were reviewed and scored by the Allred method (0-5 for proportion of positive cells, 0-3 for intensity; combined score of 0 - 8). The corresponding H&E slides were evaluated for patterns (solid, cribriform, micropapillary, papillary and clinging), nuclear grade (NG), heterogeneity in patterns and grade, apocrine features, mitoses per 10/HPF, and necrosis. Van Nuys grade was assessed as non-high grade: [low (LG) and intermediate grade (IG): NG 1,2 without and with necrosis] and high grade (HG: NG 3 with or without necrosis). Pattern heterogeneity was defined as having more than one pattern of DCIS. Grade was heterogeneous when distinct HG and non-high grade lesions coexisted. Cells with apocrine features had abundant eosinophilic cytoplasm and large round nuclei with macro-nucleoli (apocrine-type nuclei). Necrosis was quantified as comedo or punctuate.
Results: Cribriform and/or solid patterns were dominant in most cases; other patterns were rare. 42 cases had single pattern; the remainder had two or more patterns. 5 cases displayed heterogeneity of grade. Necrosis was similar in IG and HG DCIS; being more extensive in HG. Apocrine features seen in 29 were usually focal, more common in HG (41%) compared to IG (19%), and LG (17%). Diffuse apocrine features were seen in 8 (8%; 5 HG, 3 IG). 79 cases of all grades had mitoses (76%, range 0-10/hpf); 17/20 with 4 or more mitoses were HG. 79 cases (76%) were ER positive; 23/44 HG (52%, Score 0-8; median 2), 27/31 IG (87%, Score 0-8; median 8), 29/29 LG (100%, Score 7,8; median 8). ER/PR was negative in 7 diffusely apocrine DCIS (88%, 4 HG, 3 IG), were ER/PR negative. ER/PR positivity was unrelated to pattern or grade heterogeneity, focal apocrine features, necrosis or mitotic activity. The different patterns in a given case showed similar staining.
Conclusions: 1) ER/PR positivity is grade dependent with non-high grade DCIS displaying strong and diffuse ER positivity. 2) About half of high grade DCIS are ER/PR negative. 3). Heterogeneity of pattern and grade, focal apocrine features, necrosis and mitoses have no influence on ER/PR expression. 4) Diffusely apocrine DCIS appears to lack expression of ER/PR.

159 Clonality Analysis of the Peripheral Papilloma and the Cancerous Cells of the Breast

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Background: To study the clonality status of peripheral papilloma (peri-PM), ductal carcinoma in situ(DCIS) and normal tissue of the breast using an assay based on inactivation mosaicism of the length–polymorphic X–chromosomes at the androgen receptor (AR)gene site. A reliably adjutant way was explored for distinguishing the benign and malignant (or premalignant) cases in ambiguous morphologically.
Design: Twenty-six cases with peri-PM, 25 cases with peri-PM had (atypical ductal hyperplasia, ADH), 27 cases with DCIS, 16 cases with developed canceration and 20 normal breast tissues were included in the study. DNA was extracted and amplified via nested-PCR with or without previous digestion by the methylation-sensitive restriction endonuclease Hha I. The products were resolved on denaturing polyacrylamide gels and visualized through silver staining. The clonality of these samples was analyzed according to showing the lanes.
Results: Loss of polymorphism at the AR site was found in all the cases with DCIS and in 10 cases (10/25, 40.0%) with peri-PM had ADH, indicating the monoclonality of the tumor. Twenty-four cases (24/26, 92.3%) with the peri-PM and all the normal tissues were showed to be polyclonal. Among 16 cases with developed canceration, identical monoclonal alterations were observed from both components which the part of peri-PM with ADH and the part of DCIS in each case.
Conclusions: These results contributed to understand the genetic changes of the peri-PM and to confirm the peri-PM with ADH as a precancerous lesion of the breast, which it might be an important stage during the process of the precursor developing to breast carcinoma. Clonality analysis might be a useful modality to screen high-risk cases from precancerous lesions or to distinguish between the benign hyperplasia and early carcinoma.

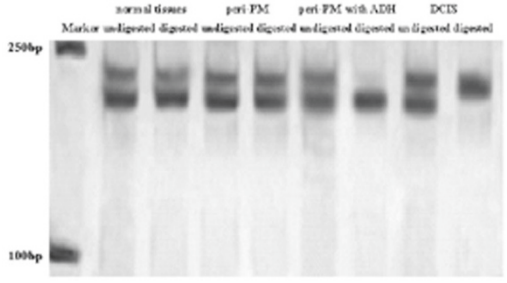


Fig 1 In the different kinds of cases: normal tissues, peri-PM, peri-PM with ADH, DCIS from polyclonal (tow bands) to monoclonal (one band)

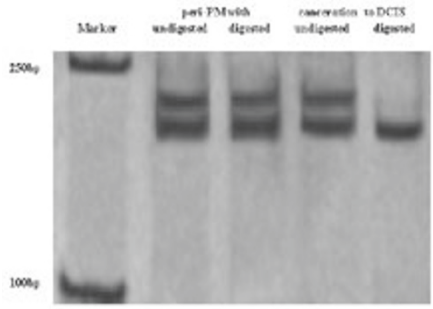


Fig 2 Different parts in a case of peri-PM with canceration to DCIS— polyclonal (tow bands) and monoclonal (one band)

160 How To Reduce the Rate of Surgical Excision Following Core Biopsies Showing Atypia?

S Nofech-Mozes, C Holloway, W Hanna. Sunnybrook and Women Health Science Centre, Toronto, ON, Canada.

Background: The aim of this study was to increase the accuracy of diagnosis of atypical ductal hyperplasia (ADH) on core biopsies to spare unnecessary surgical excisions.
Design: 176/4426 (4%) core biopsies were diagnosed as ADH; in 140 cases the results of subsequent excisional biopsy were known (2000-2204). Two breast pathologists blinded to the results of excisional biopsy reviewed all HE slides. A diagnosis of ADH was assigned using the criteria defined by Page et al. Monoclonal Ab raised against CK 5/6 (Dako clone D5/16 B4) was used for immunohistochemical staining. Cut off for positivity was >50%.
Results: On histopathology review cases were reclassified as ADH(64), UDH(44), columnar metaplasia with atypia(11) and miscellaneous benign (21). The expression of CK5/6 and the outcome of subsequent ipsilateral lumpectomy are summarized in Table 1. All 7 cases of ADH with positive CK5/6 immunostain were associated with benign disease in the subsequent excisional biopsy. Thus positive CK 5/6 immunostain has a specificity of 100% and sensitivity 67.9% in ruling out malignant disease. The probability of predicting malignancy in a lumpectomy specimen following a core biopsy increased from 43.6% to 67.8% (p=0.002) by adhering to defined criteria and using CK 5/6 immunostain (table 2). Of the 44 core biopsies reclassified as UDH, 9 cases with positive CK 5/6 had a final malignant diagnosis. Of these, 8 had other findings which were indications for excision. All 11 cases of columnar metaplasia with flat atypia were negative for CK 5/6; however, only 2 were associated with malignancy in subsequent lumpectomy.

Frequency of malignancy in surgical excision

	CK 5/6 Positive	CK 5/6 Negative	CK 5/6 not evaluated
All core biopsies n=140	10/49	48/81	3/10
ADH n=64	0/7	38/56	0/1
UDH n=44	9/35	3/6	1/3
Flat atypia n=11	0/0	2/11	0/0
Miscellaneous n=21	1/7	5/8	2/6

	% Malignancy in subsequent lumpectomy
Prior to path review n=140	43.6
ADH by path review n=64	60.9
CK 5/6 negative n=81	59.3
ADH & CK 5/6 negative n=56	67.8

Conclusions: Expertise and adherence to defined criteria are required to establish an accurate diagnosis of ADH. CK 5/6 can be a useful adjunct in cases with ADH or UDH but not in columnar metaplasia with or without flat atypia where it is universally negative. In CK 5/6 immunoreactive cases a surgical excision may not be indicated in patients without other suspicious radiological or clinical findings.

161 CISH Is an Alternative Method to FISH in Determining HER2/neu Status on Core Biopsies

S Nofech-Mozes, M Clemons, W Hanna. Sunnybrook and Women's College Helth Science Centre, Toronto, ON, Canada.

Background: Chromogenic in situ hybridization (CISH) has been technically validated recently as an accurate alternative methodology to FISH in the assessment of HER2/*neu* status in breast cancer. The aim of this study was to determine whether CISH methodology can replace FISH as confirmatory tool in core biopsies in the setting of locally advanced breast cancer (LABC) and whether CISH is equivalent to FISH in predicting response to chemotherapy.
Design: We assessed HER2/*neu* status on core biopsies in a cohort of 69 cases registered at the LABC clinic before treatment was initiated. HER2/*neu* overexpression was determined using IHC (CB11 & TAB250,). Gene amplification was studied by FISH (Vysis) and CISH (Zymed). 57 cases had complete set of data. 53 cases were invasive duct carcinoma and 5 were lobular carcinoma. Evaluation of the results was done in a blinded manner. Tumor size prior and following chemotherapy were extracted from the patient's charts to calculate the clinical response. Clinical response to chemotherapy was classified as complete (no tumor palpable), partial (tumor shrinkage >50%), stable disease (tumor shrinkage <50%) and progressive (tumor growth >25%).

Results: 20/57 (35.1%) cases were positive for HER2/*neu* gene amplification with 100% concordance between CISH and FISH results. IHC was positive for HER2/*neu* overexpression in 22/57 (38.5%) cases, with 89.4% concordance between the IHC and any of the in situ hybridization tests. There were 6 discordant cases: 4 IHC positive/ISH negative cases (3/4 were aneuploid, 1/4 had low level of amplification) and 2 case was IHC negative/ISH positive. All lobular carcinoma cases (5/57; 3 classical, 2 pleomorphic lobular) were HER2/neu negative. The correlation between HER2/neu gene amplification and the clinical response is summarized in table1.

	Clinical Responce Rate (%)				
	Complete	Partial	Stable	Progressive	Unknown follow up
Amplified	30	60	10	0	0
Non amplified	18.9	56.7	10.8	5	8

Conclusions: There is a high level of concordance between CISH and FISH in regard to HER2/neu assessment on core biopsies**.** CISH allow the use of bright field microscopy and to evaluate tumor morphology, and therefore it might be superior to FISH on core biopsies. The higher level of response in HER2/neu amplified cases provides clinical validation of the technical results and confirmed the importance of aggressive chemotherapy in HER2/neu amplified breast cancer cases.

162 *HER2* Amplification in Tubular Carcinoma of the Breast

GJ Oakley III, RR Tubbs, J Crowe, B Sebek, GT Budd, RJ Patrick, GW Procop. Marshall University, Huntington, WV; The Cleveland Clinic Foundation and the Lerner College of Medicine, Cleveland, OH.

Background: The prognostic and therapeutic implications of *HER2* gene amplification and estrogen (ER) and progesterone (PR) receptor status in breast cancer are well described, but there is a paucity of information concerning these parameters for tubular carcinomas. Therefore, we assessed the frequency of *HER2* gene amplification and the ER and PR status for 52 tubular carcinomas of the breast that were seen in a seven year experience at the Cleveland Clinic.

Design: The electronic medical records at the Cleveland Clinic Foundation were searched for all diagnoses of pure tubular carcinomas of the breast identified between January 1998 and March 2005. Fifty-two tubular carcinomas were identified. The *HER2* gene copy number was assessed by fluorescence *in situ* hybridization for the majority of tumors analyzed, whereas the estrogen and progesterone receptor status was achieved by immunohistochemistry. We also noted the presence of carcinoma *in situ*, angiolymphatic invasion, and lymph node metastases, if noted in the pathology reports.

Results: The salient features of the tubular carcinomas reviewed are presented in Table 1. None (0/52, 0%) of the tubular carcinomas demonstrated *HER2* gene amplification. Most (49/51, 95%)of the tubular carcinomas tested displayed the estrogen receptor, whereas only 55% (28/51) were positive for the progesterone receptor.

Conclusions: All of tubular carcinomas in this study failed to demonstrate *HER2* gene amplification, but were almost uniformly positive for the estrogen receptor. The frequency of *HER2* gene amplification in this unique subset of ductal carcinomas (i.e. tubular carcinomas) was significantly lower than the 15.7% frequency of gene amplification that we described in a previous study for all invasive ductal carcinomas of no special type ($p<0.01$) . We believe that if a tubular carcinoma demonstrates *HER2* gene amplification, then the pathologist should re-evaluate the histology to confirm that the correct grade was assigned.

	Molecular and Histologic Features of Tubular Carcinomas				
HER2 Gene Amplification Present	ER Positive	PR Positive	Carcinoma In Situ Present	Angiolymphatic Invasion Present	Lymph Node Metastases
0/52	49/51	28/51	37/48	0/49	5/ 25

The denominator varies depending on if the designated test was performed.

163 Activated IGF1R in Breast Cancer with Neoadjuvant Chemotherapy

G Peiró, FI Aranda, E Adrover, C Alenda, FM Peiró, A Payá. Hospital General Universitari, Alacant, Spain.

Background: Neoadjuvant chemotherapy (NACT) represents the first indication for the management of high risk group of patients with breast carcinoma (BC). Insulin-like Growth Factor (IGF) receptor system is thought to interact with several receptors such as Estrogen receptor (ER) and HER2/*neu,* in mammalian cells and BC, and regulates cell survival and proliferation. The role of activated IGF1R in BC as a predictive marker of tumor response to NACT is unknown

Design: We selected 117 core needle biopsies (CNB) and the corresponding resection specimens from BC patients in NACT with Anthracycline +/- Taxanes-containing protocols. Pathologic data from CNB and pathologic response (pR) in surgical specimens (Miller and Payne grading system) were recorded. Immunohistochemistry (IHC) for phospho-IGF1R (Tyr1131/Insulin receptor (Tyr1146) (Cell Signaling), ER, Bcl2 (DakoCytomation) and HER2/*neu* (Novocastra) was performed in CNB. Indeterminate (2+) results for HER2/*neu* were confirmed by FISH (DakoCytomation pharmaDx™). The results were scored semiquantitatively based on staining intensity (0-3+) and distribution (0-100%) (score 0-300). The relationship between activated IGF1R, pathologic and IHC data was studied

Results: The median age was 46 years (range 25-80), median clinical tumor size 57 mm (range 15-120) and post-treatment 22 mm (range 1-150). Tumors were 88% of ductal type, 5% had nuclear grade 3, 55% necrosis, 21% lymphatic invasion, associated inflammatory reaction in the tumor 12% and in the stroma 19%, and 44% had negative lymph-nodes (LN). Complete pR was seen in 17% (20/117). ER were positive (>10%) in 67% tumors, Bcl2 (>50%) in 51%, and HER2/*neu* overexpression and/or amplification in 30.7%. Higher levels of activated IGF1R (score >50; 57%) were present in ductal carcinomas ($p=0.002$), of grade 3 ($p=0.12$), with necrosis ($p=0.13$), tumor and stroma inflammatory reaction ($p=0.05$ and $p=0.18$, respectively), and cases with positive LN ($p=0.045$). Tumors that achieved complete pR showed more frequently higher expression of activated IGF1R (16/20; 80%; $p=0.022$). Negative correlation was observed between the expression of activated IGF1R and Bcl2 ($p=0.049$) and ER ($p=0.08$), and a positive trend with HER2/*neu* ($p=0.22$)

Conclusions: In our series of BC in NACT, the results suggest that activated IGF1R is a potential predictive marker of pR and LN status

164 Association of HER2/*neu* with Complete Response in Breast Carcinoma with Neoadjuvant Chemotherapy

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

Background: Patients with breast carcinoma treated with neoadjuvant chemotherapy (NACT) that achieve complete pathological response have better outcome. However, determination of molecular markers that select accurately this subset of patients are not well known

Design: Paraffin sections of core needle biopsies (CNB) from 117 BC patients in NACT with Anthracycline +/- Taxanes-containing protocols, and the corresponding resection specimens were studied. Pathologic response (pR) was graded according to Miller and Payne system. Immunohistochemistry (IHC) for HER2/*neu* (CB11; Novocastra), PTEN (Zymed), Estrogen Receptor (ER) (DakoCytomation), phospho-Akt (pAkt-Ser473) (Cell Signaling) and Ki67 (DakoCytomation) was performed. The results were scored semiquantitatively based on staining intensity (0-3+) and/or distribution (0-100%). HER2/*neu* with indeterminate (2+) results were confirmed by FISH (DakoCytomation pharmaDx™). The relationship between HER2/*neu* and pathologic features, pR and IHC results was analyzed.

Results: HER2/*neu* overexpression and/or amplification (30.7%; 35/114) was more frequently detected in tumors of ductal type ($p=0.04$), nuclear grade 3 ($p<0.000$), with necrosis ($p=0.029$), high levels of expression of Ki67 (>30%) ($p=0.009$) and pAkt (score >125) ($p=0.051$), and ER negative ($p=0.022$). However, no association was seen with PTEN (p=ns). Complete pR was seen in 17% (20/117) of the cases. Among HER2/*neu* positive tumors complete pR was achieved in 31.4% (11/35) in contrast to 11.4% (9/79) in negative tumors ($p=0.009$). Moreover, HER2/*neu* positivity correlated with negative lymph node status ($p=0.014$). Tumors containing >30% Ki67 showed higher pR ($p=0.024$), Nevertheless, no correlation was found with the levels of PTEN, ER, or activated Akt (p=ns)

Conclusions: In our series of patients with breast carcinoma in NACT with Anthracycline +/- Taxanes, HER2/*neu* positive status was a predictive marker of complete pR and negative lymph node status *Supported by grant FIS 03/1411*

165 Comparison of Different Commercial *In Situ* Hybridization (ISH) Kits for HER-2 Testing in Breast Cancer. Looking for the Accurate Cut Off

FM Penault-Llorca, F Mishellany, N Lagarde, A Cayre. Centre Jean Perrin, Clermont-Ferrand, France.

Background: Herceptin was previously approved in Europe for use in metastatic breast cancer patients whose tumours were tested as HER2-positive (so called "HER2 overexpression") only by the immunohistochemistry (IHC) testing method. Eligibility for Herceptin treatment can now be determined by tumours having either HER2 overexpression (IHC) or HER2 gene amplification, namely by fluorescence in situ hybridisation (FISH) and chromogenic in situ hybridization (CISH). But discrepancies can occur between the results because of variability between the kits used. The aim of the study was to compare the results of amplification determined either by CISH or by FISH using 4 commercially available kits

Design: Fifty five patients with invasive breast carcinoma, formalin fixed and paraffin embedded (FFPE) were included in this study. Cases were selected in order to have representative proportion of 2+ immunohistochemistry cases. For each test, we have compared the lengh and complexity of the technique, interpretation pitfalls using the cut off recommended by the suppliers. We evaluated 2 IHC tests - In house tests and Herceptest (Dakocytomation) and 4 ISH tests - dual probe: Path Vysion HER2 kit Vysis/Abbot, HER2 FISH pharmDx kit Dakocytomation, single probe: INFORM HER2 test Ventana and CISH: Zymed HER2 CISH.

Results: 33% tumors were 3+ with the in-house test, and 23: 2+. Amplification was observed in 31% scored as amplified with Path Vysion kit, 33% with pharmDx and 64% with INFORM (cut off>4). With CISH (cut off >=6), 36% tumors showed HER2 gene amplification. Path Vysion HER2 kit was considered as the gold standard. Agreement was excellent (100%) between the two dual probe (Abbot and Dako) and CISH (94%)and moderate with INFORM (70%). For in-house IHC, there is an agreement of 100 % for negative cases, and of 94 % for 3+ cases. For INFORM, if we apply a new cut off of 6 spots then, the agreement with PathVysion was of 96%.

Conclusions: Therefore, we conclude that CISH is a methodology that could be used as an alternative to FISH in the HER2 testing algorithm.Our results showed the accuracy of CISH for the evaluation of HER2 status. We have also showed that, with a proper cut-off, the INFORM kit was also quite comparable to dual probe kits. We recommend the use of CISH as an alternative to FISH in the HER2 testing algorythm. CISH is more adapted to pathology labs and more convenient to interpret than FISH. Dual probe FISH kits remain useful for the rare borderline cases.

166 Intraoperative Frozen Section Analysis of Sentinel Lymph Nodes in Breast Cancer: A Retrospective Review of 274 Cases. What Improvement Is Expected from Intra-Operative Real-Time RT-PCR?

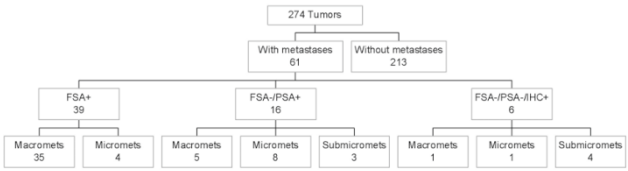
TT Pham, N Weidner, A Shabaik. University of California, San Diego, CA.

Background: Intraoperative frozen section analysis (FSA) of sentinel lymph nodes (SLN) is sensitive and specific and has been widely used for detecting subclinical breast cancer lymph node metastasis. Recently, intraoperative reversed transcriptase polymerase chain reaction (RT-PCR) has emerged as a potentially more sensitive method. The aim of this study is to review our institutional experience with SLN FSA in breast cancer and to review the literature in assessing the expected gain in sensitivity from RT-PCR.

Design: Breast cancer cases with SLN FSA were retrieved. SLN analyses (FSA, PSA, and immunohistochemistry [IHC]) were abstracted from pathology reports. The

sensitivity and specificity of FSA is compared with PSA/IHC. Published rates of SLN RT-PCR were compiled.
Results: From 2001 to 2005, 274 breast carcinomas with SLN FSA from 265 patients were performed. 228 tumors were invasive; 46 were exclusively in-situ. SLN metastases were detected in 61 cases; all but two were with invasive tumors. Figure 1 depicts the detection method and size of metastases. Compared with the PSA/IHC results, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of FSA were 64%, 100%, 97%, 91%, and 92%, respectively. These rates were comparable with those of 28 previous studies. In our study, 16 of 22 false negative cases were micro- or submicrometastases. There were no additional metastases in 5 of 9 micrometastatic cases that underwent complete axillary dissection. RT-PCR on fresh frozen SLN tissues in 10 studies yielded average sensitivity, specificity, PPV, NPV, and accuracy rates of 65%, 69%, 56%, 81%, and 67%, respectively, when compared to PSA/IHC controls.
Conclusions: Our experience confirms that intraoperative FSA is highly specific and sensitive for detecting lymph node metastasis in breast cancer. The published RT-PCR results show no appreciable sensitivity improvement over FSA. Because of its current low specificity, the significance of a positive RT-PCR result is uncertain. At present, SLN RT-PCR should be regarded as an investigational tool and not as a replacement for SLN FSA.

Figure 1. Detection method and size of SLN metastases in 274 breast carcinomas



167 Accuracy of Touch Prep Diagnosis of Breast Cancer Sentinel Lymph Nodes

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Background: Sentinel lymph node (SLN) biopsy is an accurate staging procedure for breast cancer. Intraoperative analysis of SLN allows completion axillary lymph node dissection (ALND) when the SLN is positive. Given the morbidity of ALND, false negative (FN) results are acceptable. We reviewed our experience with SLN biopsy and intraoperative interpretation, specifically focusing on histologic parameters associated with FN and FP results.
Design: The surgical pathology material from cases in which FN and FP diagnoses were rendered was reviewed. All touch preps from these cases were retrospectively analyzed by immunocytochemistry for cytokeratin expression. Pathologic characteristics of metastases were evaluated. Statistical significance between tumor characteristics and TP diagnostic accuracy was determined by paried t-test or analysis of variance with *P* value of 0.05 considered significant.
Results: 169 patients underwent SLN mapping and biopsy by a single surgeon in our institution between July 1999 and April 2004. Of these 115 were truly negative and 35 truly positive, by comparison with permanent sections. There were 15 FN cases, 2 FP cases, and in 3 cases the touch prep was called suspicious, but no axillary dissection was performed. Touch prep diagnoses had a sensitivity of 70%, specificity of 98%, positive predictive value was 95%, and negative predictive values of 89%. Falsely negative cases correlated with the size of the largest nodal metastasis (6.6 +/-4.4 mm in TP vs 2.1 +/-1.7mm in FN) cases as well as the number of involved nodes 3.5 =/-2.3 in TP vs 1.4 +/-1.1 in TN ($P<0.05$). None of the FP touch preps nor the 3 touch preps called suspicious contained any cytokeratin-expressing cells when immunocytochemical analsyis was performed. In contrast, six of 15 FN cases had small clusters cytokeratin-positive cells. When the FN patients underwent completion ALND, none had additional positive lymph nodes.
Conclusions: Intraoperative touch prep analysis of SLN is accurate in most cases. Elimination of FP diagnoses can be assured by diagnosing as positive only clear cut examples. Understanding by the surgeon and patient that a false negative rate higher than would be acceptable in other intraoperative settings is appropriate. False negative cases occur in the setting of micrometastatic disease, with the high likelihood that additional lymph nodes will not harbor further metastases.

168 Immunohistochemical Phenotype of Invasive Breast Carcinoma in Young Women. Correlation with Cox-2 and PTEN Expression

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Background: Previous studies have shown that immunohistochemistry can classify breast carcinomas into estrogen receptor (ER)+/luminal, normal breast-like, Her-2 overexpressing, and basal-like subgroups (cytokeratin 5/6 and/or Her-1 +). The aim of our study was to define subtypes of invasive breast carcinoma (IBC) in a series of patients ≤35 years of age and to correlate the results with Cox-2 and PTEN immunohistochemical expression.
Design: A total of 53 women ≤35 years with IBC were selected. To identify an immunohistochemical profile we examined the expression of CK5/6 (Dakocytomation), estradiol and progesterone receptors (ER/PR) (Dakocytomation), Her-1/EGFR (Dakocytomation) and Her-2/neu (CB11; Novocastra) in a tissue microarray block (cylinders of tissue of 1 mm diameter). Cases were classified as: (a) basal-like (CK5/6 and/or Her-1 positive), (b) Her-2 (positive), (c) luminal (RE/RP positive) and (d) indeterminate (Her-2/RE/RP/Her-1/CK5/6, negative). These tumor phenotypes were correlated with Cox-2 (Cayman Chemical) and PTEN (Zymed) expression.
Results: Age of the patients ranged from 15 to 25 years (mean 30). Her-1 positivity (≥10%) was observed in 11%, CK5/6 (≥10%) in 7.5%, RE (≥10%) in 61%, RP (≥10%) in 57% and Her-2 (3+) in 9.4%. Seven cases (13.2%) were classified as basal-like type, 5 (9.4%) as Her-2/neu (positive), 36 (67.9%) luminal, and 5 (9.4%) indeterminate. Cox-2 expression (≥10%) was more common in basal-like tumors (100%), compared with 40% in Her-2 (positive) and 17% of luminal type ($p<0.000$). Regarding PTEN, loss of expression (<10%) was observed in 71% of basal-like tumors, 40% of Her-2 (positive) and in 25% of luminal type ($p=0.004$).
Conclusions: In the present series of IBC in young patients (≤35 years), we found predominantly the luminal subtype (67.9%) followed by the basal-like subtype (13.2%). Moreover, basal-like tumors showed increased Cox-2 and loss of PTEN expression.

169 Breast Cancers with Polysomy Chromosome 17: Its Frequency and Effects on the Ploidy and HER-2/neu Expression

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Background: Proto-oncogene, *HER-2/neu,* is an important biomarker in breast cancer and its amplification is correlated with poor prognosis. *HER-2/neu* is located on chromosome 17. Specific treatment protocols are available to breast cancer patients with amplified *HER-2/neu* gene. Polysomy of chromosome 17 is observed occasionally during *Her-2/neu* testing by FISH. This complicates scoring of *Her-2/neu* amplification in relation to the ratio of Her-2/neu signals to chromosome 17 signals. The current study attempts to evaluate the frequency of polysomy 17 in breast cancers and characterize the effect of polysomy 17 on the DNA ploidy by flowcytometry and Her-2/neu status by immunohistochemistry (IHC) in breast carcinomas.
Design: A total of 155 cases were analyzed for HER-2/neu status by IHC and FISH and ploidy status by flowcytometry. These cases were selected for data analysis after IRB approval. IHC, FISH and Flow studies were performed at Women & Infants Hospital using FDA approved Dako Cytomation reagent for IHC (HercepTest®), VYSIS (PathVysion®) detection system for FISH and Becton-Dickinson FACSCan Flow Cytometer. IHC was scored according to the manufacturer's guidelines as 0-1+ (negative), 2+ positive and 3+ positive.
Results: Nine of 155 (~6 %) cases had polysomy of chromosome 17. All nine were invasive ductal carcinomas, 4 had Modified Bloom-Richardson grade II of III and 5 had grade II of III. DNA ploidy studies were performed in 8 of the 9 cases. Of the 8 cases, 6 (75%) were aneuploid and remaining two were diploid tumors. One tumor did not have sufficient tumor volume to perform ploidy study. All 9 cases had 2+ Her-2/neu expression by IHC. None of the nine cases show *Her-2/neu* amplification by FISH.
Conclusions: The frequency of polysomy 17 in breast carcinomas appears to be approximately 6%. Of these, approximately three quarter of the cases were found to be aneuploid by DNA ploidy studies. All these cases had 2+ IHC HER-2/neu expression and none of these cases show gene amplification by FISH. It appears that IHC studies show increased expression of HER2/neu protein due to the presence of increased number of *HER2/neu* gene copies due to Polysomy 17. Although *HER-2/neu* gene is not amplified within each chromosome 17, breast cancers with polysomy 17 may have clinical significance due to increased expression of Her-2/neu protein. It may be prudent to treat this subset of breast cancer cases similar to bona fide *HER-2/neu* amplified cases.

170 Correlation of Her2/neu Overexpression (IHC) with Gene Amplification (FISH), and Its Relation to Chromosome 17 Aneuploidy: A 5 Year Experience with Invasive Ductal Carcinoma

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Background: Her2/neu oncogene shows overexpression and/or amplification in approximately 20%-30% of breast cancers and has been associated with a poor prognosis and response to trastuzumab therapy. Although Her2/neu gene status determines the eligibility of breast cancer patients for trastuzumab therapy, only a fraction of these patients respond to targeted therapy, indicating the role of other factors. Several studies have related the increased expression of Her2/neu to increased copy number of chromosome 17 rather than amplification of the Her2/neu gene. We compared the IHC and FISH results in invasive ductal carcinomas (IDC) according to histologic grades, and determined the frequency of chromosome 17aneuploidy (chr 17 Aneu) associated with discordant results.
Design: 390 cases of IDC diagnosed from 2000 to 2005 were included in the study only if IHC (DAKO Hercept Test) and FISH (Vysis PathVysion) results were available. Tumors were classified as IDC and graded according to the Bloom-Richardson system.

Results:

Results

Grade	Her2/neu IHC		FISH	Chr 17 Aneuploidy
Grade1 (n=87)	negative	21%	11% **(amp)**	50%
			89% (nonAmp)	44%
	2+	37%	9%**(amp)**	66%
			91% (nonAmp)	45%
	3+	42%	41% **(amp)**	7%
			59% (nonAmp)	50%
Grade II (n=132)	negative	17%	9% **(amp)**	0%
			91%(nonAmp)	33%
	2+	42%	14% **(amp)**	0%
			86% (nonAmp)	52%
	3+	40%	62%**(amp)**	3%
			38% (nonAmp)	55%
Grade III (n=171)	negative	30%	6% **(amp)**	0%
			94% (nonAmp)	33%
	2+	24%	26% **(amp)**	0%
			74%(nonAmp)	45%
	3+	46%	74% **(amp)**	2%
			26%(nonAmp)	30%

Conclusions: Grade I and II Invasive ductal Ca with negative to 2+ Her2/neu by IHC showed no amplification by FISH in 86-91%. Grade III IDC 3+ by Her2/neu IHC were associated with concordant results by FISH in 74%; 30% non amplified showed chr 17 aneuploidy. Grade II 3+ cases and Grade III 2+ cases showing no amplification by FISH, were associated with chr 17 Aneu in approximately 50%. Thus, chr 17 Aneu appears to play an important role in cases with discordant results between IHC and FISH.

171 Her2/neu Expression (IHC), Amplification (FISH), and Chromosome 17 Aneuploidy in Invasive Lobular Carcinoma

A Radhakrishnan, A Nassar, M Lewis, C Phillips, C Cohen. Emory University Hospital, Atlanta, GA.

Background: Invasive lobular carcinoma (ILC) comprises approximately 10% of breast cancers and appears to have a distinct biology. Because its incidence is less than infiltrating ductal carcinoma, few data have been reported that address the correlation of immunohistochemistry (IHC) and Her2/neu status. The presence of HER2/neu gene amplification is prognostically and therapeutically significant for patients with breast cancer. We sought to determine whether a relationship exists among HER2/neu status by IHC, gene amplification by *FISH*, and chromosome 17 aneuploidy (chr 17 Aneu) in ILC.

Design: 180 ILC, diagnosed between 2000-2005, were studied for Her2/neu expression by IHC (DAKO Hercept test) and amplification by FISH (Vysis PathVision), chr 17 Aneu, and Estrogen Receptor(ER) status. ILC were classified based on Bloom Richardson's system.

Results:

Immunohistochemistry results in Invasive Lobular Breast carcinoma

ER IHC	Her2/neu IHC			
ER positive =96%(n=173)	57% (n=98)	9% (n=17)	21% (n=36)	13% (n=22)
ER negative =4%(n=7)	71% (n=5)	0% (n=0)	0% (n=0)	29% (n=2)

	ER + & Her2/neu- (2)		ER + & Her2/neu1+ (2)		ER + & Her2/neu 2 + (27)		ER +& Her2/neu 3+ (4)	
FISH (n=36)	0% (n=0) **Amp**	100%(n=2) Non- **Amp**	0% (n=0) Amp	100%(n=2) Non- **Amp**	0% (n=0) Amp	100%(n=2 7) **Non Amp**	25% (n=1) **Amp**	75% (n=3) **Non Amp**
Chr 17 Aneu (n=12)	0% (n=0)	0% (n=0)	0% (n=0)	50% (n=1)	0% (n=0)	37% (n=10)	0% (n=0)	33% (n=1)

Conclusions: 1.In Invasive Lobular carcinoma, Her2/neu amplification by FISH was seen in only one of four Her2/neu IHC 3+ positive cases. 2.There was no Her2/neu FISH amplification seen in IHC negative, 1+, or 2+ positive Her2/neu. 3.One third of the ILC cases 2+ and 3+ by IHC were associated with chr17 Aneu. 4. The Majority (96%) of the ILC were ER positive; only 13 % were Her 2/neu 3+ positive by IHC. 5.Thus, chr 17 Aneu may play an important role in ILC with discordance between IHC and FISH results regarding Her2/neu status.

172 Distribution of Nerve Growth Factor Receptor 1 (NGFR1): A Novel Myoepithelial Marker Expressed in a Subset of Basal-Like Breast Carcinomas with Good Prognosis

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Background: Nerve growth factor receptor (NGFR) is transmembrane glycoprotein without intrinsic tyrosine kinase activity, whose expression is not restricted to neural cells. NGFR is reported to act as a tumour suppressor, negatively regulating cell growth and proliferation.

Design: The distribution of NGFR was immunohistochemically analysed in normal breast tissue and in 141 benign and preinvasive breast lesions, in 20 metaplastic breast carcinomas and in two cohorts of breast cancer patients: a series of 245 invasive breast carcinomas of usual type and 37 high grade, basal-like breast carcinomas.

Results: Immunohistochemistry demonstrated that NGFR consistently displayed membrane reactivity in myoepithelial cells arranged as a continuous layer around normal ducts and lobular units, intralobular fibroblasts, perivascular stromal cells and nerve bundles. Myoepithelial cells of benign proliferations and pre-invasive lesions were consistently positive for NGFR. Scattered NGFR positive cells were observed in solid areas of 6 out of 9 hyperplasias of usual type, whereas in flat atypia, lobular carcinoma in situ and all but one DCIS (2.5%), NGFR was restricted to the myoepithelial layer. Eleven out of 245 (4.5%) usual types of breast cancer, 9 out of 20 (45%) metaplastic breast carcinomas and 14 out of 37 (38%) basal-like breast carcinomas showed positivity for NGFR. NGFR expression in invasive tumours significantly correlated with that of cytokeratins 5/6 ($p<0.05$), 14 ($p<0.0001$) and 17 ($p<0.0005$) and EGFR ($p<0.0001$) and displayed an inverse correlation with oestrogen and progesterone receptors ($p<0.0001$ and $p<0.0001$, respectively). NGFR showed a statistically significant association with longer disease-free ($p<0.05$) and overall survival ($p<0.01$) in the cohort of patients with basal-like carcinomas.

Conclusions: This study demonstrates the utility of NGFR as a new adjunct marker to identify myoepithelial cells in preinvasive lesions and myoepithelial differentiation in breast carcinomas. Furthermore, our data suggest that NGFR may identify a subgroup of basal-like breast carcinomas with good prognosis.

173 Evaluation of the Relationships between pAkt and Beta-catenin in Invasive Breast Carcinoma

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Background: The phosphatdylinositol 3' kinase (PI3K)/pAkt pathway is implicated in many aspects of tumor progression; including cell survival, invasion, and metastasis. Metastasis and invasion are complicated processes, which include cell dishesion, migration and lymphatic and vascular invasion. The specific downstream effectors of pAkt that are involved in these processes have yet to be fully elucidated. Recent data has demonstrated that activation of the PI3K/pAkt/I kappa B kinase (IKK) pathway upregulates beta-catenin. Beta-catenin is an important transcription regulator, which is implicated in tumor angiogenesis and metastasis in various human carcinomas (Agarwal *et al*, Oncogene 2005). The present study hypothesizes a relationship between pAkt, beta-catenin and E-cadherin.

Design: Formalin-fixed, paraffin-embedded tissue from 210 (80 cases with lymph node metastasis and 130 cases without lymph node metastasis) cases of invasive breast carcinoma were retrieved from the archives of the Department of Pathology at Baystate Medical Center. Eighteen tissue microarrays (TMAs) were created. Five 0.8mm cores were taken from separate areas of each tumor. The expressions of the following regulatory proteins were evaluated using immunohistochemical analysis: pAkt, Beta-catenin and E-cadherin (Cell Signaling Technology). The immunohistochemistry was performed on a Dako automated platform.

Results:

pAkt, Beta-catenin and E-cadherin expression

	pAkt +	pAkt -	B-Cat +	pAkt +/ B-Cat +	B-Cat+/ E-Cad-	pAkt+/B-Cat+/E-Cad-
ICA c +LN: 80	41/80, 51%	39/80, 49%	63/71*, 89%	34/71*, 48%	16/71*, 23%	5/71*, 7%
ICA c -LN: 130	60/130, 46%	70/130, 54%	101/119*, 85%	56/119*, 47%	27/119*, 23%	12/119*, 10%
Total: 210	101/210, 49%	109/210, 51%	164/190*, 86%	90/190*, 47%	43/190*, 23%	17/190*, 9%

* Evaluation of B-catenin was not performed on 20 cases.

Conclusions: No relationship was demonstrated, either singly or in combination, between pAkt, beta-catenin, and E-cadherin. pAkt regulation of beta-catenin/E-cadherin expression does not appear to play a significant role in invasive breast carcinoma.

174 Evaluation of the PI3K/pAkt Pathway in Breast Carcinoma Which Are Negative for ER, PR and HER-2/*neu* Receptor Expresssion

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Background: In breast carcinomas which are negative for ER, PR and HER-2/*neu* receptor, triple negative, hormonal or Herceptin therapy is generally not indicated the treatment options are limited. The PI3K/pAkt pathway has been shown to be activated in a variety of human neoplasms, including breast carcinoma. pAkt is central to numerous receptor tyrosine kinase (RTKs) activated pathways, providing alternate mechanisms of downstream upregulation of regulatory proteins associated with tumorogenic properties. Tyrosine kinase inhibitors and signal transduction modulators targetting the PI3K/pAkt pathway have shown significant promise in preclinical trials when they are used in combination with standard treatment modalities (ie. chemotherapy and radiation therapy). Evaluation of key regulatory proteins in the PI3K/pAkt pathway in order to elucidate potential targets for future therapy in the "triple negative" breast carcinomas.

Design: Formalin-fixed, paraffin-embedded tissue from 19 triple negative cases of invasive ductal carcinoma (IDC) of the breast, were retrieved from the archives of the Department of Pathology at Baystate Medical Center. Tissue microarrays (TMAs) were created using five 0.8mm cores taken from separate areas of each tumor. The expressions of the following proteins were evaluated using immunohistochemical analysis: EGFR, PDGFR, VEGFR, PI3K, phospho-mTOR and pAkt (Cell Signaling Technology). The immunohistochemistry was performed on a Dako automated platform.

Results: pAkt expression was present in 8 of 19 (42%) of cases. Six of these eight cases (75%) demonstrated coexpression of a receptor tyrosine kinase (RTK): EGFR (4), PDGFR (1) and VEGFR (1). EGFR was over-expressed in 9 of 19 (47%) cases. Phospho-mTOR was present in 4 of 19 (21%). Three cases with overexpression of phospho-

mTOR also co-expressed pAkt (3 of 4, 75%) . Combined overexpression of PI3K, pAkt and phospho-mTOR was present in 2 of 19 (10.5%) cases.
Conclusions: The PI3K/pAkt pathway is activated in a major subset (42%) of triple negative breast carcinomas. Evaluation of RTKs and downstream mediators of the PI3K/pAkt pathway in triple negative breast carcinomas may identify a subset of patients who would benefit from targetted therapy directed towards key proteins in this regulatory pathway (subsequent statistical analysis to be performed).

175 Hyperphosphorylation of Translational Repressor 4E-BP1 in Human Breast Is a Prognostic Factor

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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 translation machinery via activation of the p70S6 kinase and via inhibition of eIF4E inhibitor 4EBP1, and constitutes a main controller in cell growth. mTOR downstream proteins 4EBP1 and eIF4E play a crucial role in regulating translation and progression in cell cycle by control of cyclin D1 and c-myc. As described in *in vitro* models, 4EBP1 can also be phosphorylated by other signaling pathways as Ras-Raf-MAPK.
Design: We have analyzed 103 paraffin-embedded human breast tumors with a complete IHC profile including membrane receptors and phosphorylated (p) proteins: HER2, EGFR, p53, ER, PR, p42/44MAPK, Akt, 4EBP1, eIF4E, p70S6K, S6 and Ki67. Levels of expression were evaluated as percentage and intensity of stained cells (Hscore).
Results: Activation of PI3K/Akt/mTOR signaling cascade was significantly detected in a high proportion of breast tumors (41.9%). Patients with HER2 overexpression showed a higher activation of Akt compared to negative ($p<0.001$) and levels of pAkt were correlated with its downstream molecules p4EBP1 ($p=0.001$) and pp70S6K ($p=0.05$), and p4EBP1 was correlated with its downstream protein peIF4G ($p=0.012$). Whereas up to 81.5% of tumors expressed p4EBP1 and in a proportion of them (55.3%) was not detected an upstream activation. Interestingly, p4EBP1 was mainly expressed in poor differentiated tumors ($p<0.001$), significantly correlated with tumor size ($p<0.001$) and with presence of lymph node metastasis ($p=0.002$). Co-expression of p4EBP1 and peIF4G correlated with high tumor proliferation rate ($p=0.012$). Finally, majority of tumors with p4EBP1 showed an increased rate of locoregional recurrence ($p=0.002$).
Conclusions: In breast cancer, activation of major cellular signaling pathways is partially mediated by overexpression of membrane erbB receptors, but frequently, activation of signaling proteins is not just dependent of these receptor signals. Evaluation of activation of the converging downstream signaling proteins, as 4EBP1, could be a stronger prognostic indicator regardless upstream oncogenic alterations. In this study, hyperphosphorylation of 4EBP1 in breast cancer is strongly associated with high grade, tumor size, lymph node metastasis and locoregional recurrences.

176 The Expression of Epithelial Membrane Antigen and D2-40 in Invasive Micropapillary Carcinoma of the Breast – Role in Pathogenesis

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Background: Invasive micropapillary carcinoma is an aggressive variant of invasive duct carcinoma of the breast that morphologically mimics ovarian serous carcinoma. Staining with MUC1 has demonstrated a possible reverse polarization phenomenon in these tumors. D2-40 (lymphatic marker) and WT-1 (marker of serous ovarian carcinoma) have not been previously examined in micropapillary carcinomas. Previous studies of ER/PR and Her2/neu status have shown variable results.
Design: We reviewed 91 cases of invasive micropapillary carcinoma seen at our institution in the last 5 years. Immunohistochemical (IHC) staining was performed for WT-1 and D2-40 in 56 cases, EMA in 45 cases, CD31 in 51 cases, ER and PR in 82 cases, and Her2/neu oncoprotein (Tab250 and CB11 antibodies) in 81 cases. Cases with equivocal IHC results for Her2/neu were further tested with FISH methodology using the Vysis probe.
Results: EMA showed a predominately linear staining on the stroma-facing aspect of the cells in 27 out of 45 cases, while 18 showed predominately cytoplasmic and/or luminal staining. D2-40 focally stained the lining of spaces surrounding tumor cells in 13/54 (23%) cases. CD31 showed focal staining in 4/51 (8%). WT-1 showed nuclear positivity in 11/56 cases (20%). ER was positive in 62/82 (76%), PR in 49/82 (60%) and Her2/neu overexpression/amplification was present in 24/81 (30%) cases.
Conclusions: EMA staining in most cases was linear and stroma-facing, indicative of a reverse polarization phenomenon, but many cases demonstrated cytoplasmic staining which could be related to internalization of the cell membrane, a phenomenon commonly seen in breast carcinoma. The positivity for D2-40 and CD31 around some of the spaces containing tumor cells indicates focal lymphovascular invasion. Our findings suggest that the phenotype of invasive micropapillary carcinoma is due mostly to a reverse polarization phenomenon, with intratumoral lymphovascular invasion as a minor component. Positivity for WT-1 in eleven cases (20%) highlights the importance of considering breast and not only ovary as the primary site for metastatic papillary carcinoma. This study confirms the high percentage of positivity of ER and PR in spite of the aggressive behavior of this tumor. Our study also showed that only 30% of the tumors were Her2/neu positive which is significantly lower than previously reported. This is likely due to the current standardized methodology and scoring for Her2/neu testing and the use of FISH for equivocal cases.

177 Human Mammary Stroma Modulates Mammary Epithelial Proliferation in 3D-Matrix

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Background: There is increasing evidence that the stroma can modulate the tumorigenic potential of malignant mammary epithelia. However, the specific effects of stroma on pre-malignant mammary epithelia have not been investigated. Fifty percent of human breast carcinomas express elevated levels of normal Ha-Ras protein, and expression of oncogenic Ras is one of the components required for transformation of human mammary epithelial cells (HMEC). We have utilized HMEC expressing oncogenic Ha-Ras (HMEC-Ras) as a model of pre-malignant epithelia to study the effect of stroma on cellular events associated with tumor progression, such as morphology and proliferation. We hypothesize that normal, high-risk (BRCA1-HMF) and carcinoma-associated human mammary fibroblasts (CAF) differentially modulate mammosphere morphology and proliferation in 3D co-cultures when compared to normal, low-risk human mammary fibroblasts (HMF).
Design: To test our hypothesis, different HMF were embedded in a thin layer of Matrigel and epithelial cells were seeded on top at a 3:1 fibroblast:epithelial cell ratio. The effect of the different HMFs on HMEC and HMEC-Ras were assessed by analyzing 1) mammosphere formation and morphology and 2) cellular proliferation by confocal imaging. Ki-67 immunostaining was utilized as a marker for proliferation.
Results: The preliminary results indicate that both HMEC and HMEC-Ras form mammospheres when cultured in 3D matrigel, however, the mammospheres formed by HMEC-Ras are larger and lack a lumen. In addition, when co-cultured with HMF, both HMEC and HMEC-Ras form interconnected duct-like structures. With respect to proliferation, both BRCA1-HMF and CAF, but not normal, low risk HMF increase the proliferation of HMEC and HMEC-Ras when co-cultured in 3D-matrigel. These experiments were conducted in triplicate and representative images are presented. Each of the HMF utilized was derived from one individual.
Conclusions: We conclude that cross-talk between mammary epithelia and both BRCA1-HMF and CAF promote the proliferation of mammary epithelium and may thus facilitate the tumorigenicity of these cells.

178 Lack of EGFR Gene Amplification for Metaplastic and Basal-Like Breast Cancers Measured Using Dual-Probe FISH

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Background: The human epidermal growth factor receptor protein (EGFR or HER-1) has been shown to be overexpressed in approximately 10-20% of breast cancers and levels of EGFR expression are thought to be increased in basal-like and metaplastic type cancers. Basal-like tumors possess a "triple negative" immunophenotype (ER-/PR-/HER2-) and frequently express cytokeratin 5/6. Metaplastic breast cancers display a similar immunoprofile and are thought to belong to the basal-like breast cancer group. In addition to its pathobiological role, EGFR protein may also prove to serve as a therapeutic target for these tumor types. The best method of EGFR status determination for breast cancer is currently unclear as are the specific biological mechanisms responsible for its overexpression. For this study we sought to measure EGFR gene and protein levels using dual-probe fluorescence in-situ hybridization (FISH) and immunohistochemistry (IHC), respectively, in a cohort of metaplastic and basal-like breast cancers.
Design: Whole tissue sections from 25 breast cancer cases were used for study (18 metaplastic and 7 basal-type). EGFR IHC and dual-probe EGFR FISH (Vysis) were performed on each case. For EGFR IHC scoring, a HER2 scoring system (0,1+,2+ and 3+) was used. Cases with IHC scores of 2+ and 3+ were considered positive for overexpression. For FISH scoring, the ratio of LSI-EGFR (EGFR gene copy number) to CEP7 (chromosome 7 number) was used. 20-60 nuclei from each case were scored and LSI-EGFR to CEP7 ratios equal to or above 2.0 were considered positive for EGFR gene amplification.
Results: Overall, 13/25 cases (52%) showed either 2+ or 3+ levels of EGFR protein expression. Specifically 5/7 basal-like (71%) and 8/18 metaplastic cancers (44%) showed EGFR overexpresion. None of the cases tested were found to have EGFR gene amplification using a LSI-EGFR to CEP7 ratio of 2.0 as the cutoff. Ratios ranged from 1.0 to 1.5 and chromosome 7 polysomy was observed in 13 cases. Polysomy was observed in 4/12 EGFR protein negative cases and in 9/13 EGFR protein positive cases.
Conclusions: Basal-like and metaplastic breast cancers have increased rates of EGFR protein overexpression compared to those observed for breast cancer in general. EGFR gene amplification does not appear to play an important role in protein overexpression in this cohort of breast tumors. Alternatively, dual-probe FISH with use of a LSI-EGFR to CEP7 ratio may not be an effective means of detecting cases with potentially clinically significant low-level gene amplification.

179 Laminin-5 Expression in Basal/Myoepithelial-Like Mammary Carcinoma

CL Schiller, WR Wiseman, C Luan, WG Watkin, SM Sitterding, EL Wiley, MZ Gilcrease, LK Diaz. Northwestern University, Chicago, IL; UT MD Anderson Cancer Center, Houston, TX.
Background: Laminin-5 is not only a basement membrane protein but also the principal ligand for the $\alpha 6\beta 4$ integrin, a cell surface receptor shown to play an important role in tumor cell migration and invasion. Laminin-5 is expressed in 25% of patients with early breast cancer, and cDNA microarray analysis of invasive breast carcinomas has demonstrated that laminin-5 clusters with genes characteristic of basal/myoepithelial cells in these tumors. In the current study we sought to determine whether laminin-5 protein expression serves as a marker for basal/myoepithelial-like mammary cancers by examining its expression in a group of these tumors, including a large proportion of metaplastic breast carcinomas.

Design: Using previously published criteria we identified 50 cases of basal/myoepithelial-like breast carcinoma, including 29 cases of metaplastic breast carcinoma. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded whole tissue sections from these 50 cases using automated techniques and a commercially available monoclonal antibody to the γ2 chain of laminin-5 (Chemicon; Temecula, CA). Staining was scored as: 0 (< 5% staining), 1+ (5% to 19% staining), and 2+ (20% or greater staining). Positive staining patterns included: peri-tumoral, cytoplasmic, and tumor-stromal interface staining.
Results: Laminin-5 expression in this group of basal/myoepithelial-like mammary carcinomas was observed in 38/50 cases (76%), including 29/50 cases (58%) with 2+ staining. In positive cases, a mixture of all three staining patterns (peri-tumoral, cytoplasmic, and tumor-stromal interface staining) was observed.
Conclusions: A markedly higher rate of laminin-5 expression was observed in this group of basal/myoepithelial-like mammary carcinomas compared to our previously studied cohort of early stage breast cancers (76% versus 25%, respectively). Together with previous cDNA microarray data showing clustering of the laminin-5 gene with other genes characteristic of basal/myoepithelial-like carcinomas, our findings suggest that laminin-5 (perhaps in combination with another basal/myoepithelial cell marker) may be a useful marker for this aggressive subtype of breast cancer. As the ligand for α6β4 integrin, laminin-5 may also be a predictive marker to select patients for therapies targeting the α6β4 integrin signaling pathway.

180 Outcome of Benign Papillary Lesions of the Breast Diagnosed by Needle Core and Mammotome Biopsies with 3 to 5 Year Follow Up

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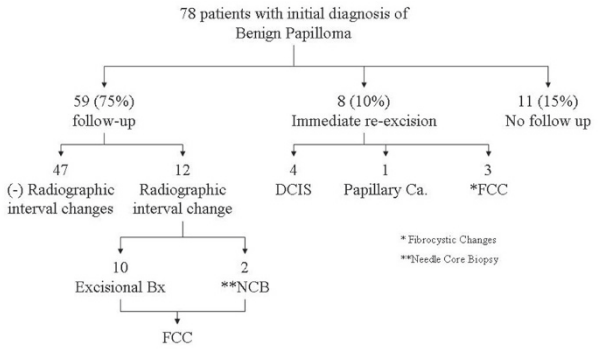
Background: Management of benign papillary lesions (BPL) diagnosed by 11 to 14 gauge needle core biopsies (NCB) or mammotome biopsies (MB) remains controversial, even when the lesions are small (≤1cm) and considered radiologically completely excised at initial biopsy (Bx).
Design: To assess the need for immediate re-excision, a series of 78 patients with initial diagnosis of BPL dating from 12-2000 to the present, were followed by mammography and/or ultrasound (US) examination. The following parameters were recorded: Initial mammographic findings: mass and/or microcalcifications, size of the lesions, number of mammographic follow-up controls, length of follow up, types of hyperplasia within the lesions, and subsequent re-excision diagnoses. Patients in which the initial Bx results did not correlate with the radiographic findings were immediately re-excised, as well as patients with interval follow up changes.
Results: All patients had new non-palpable lesions detected either by mammogram or US screening. Radiographically, 68 (87%) of the patients presented either with indeterminate microcalcifications (Ca++), or masses ranging from 0.4 to 1.3 cms (47 and 31% respectively). All but 8% of the patients had 4 to 5 year mammographic follow up with an average of 3.7 mammograms per patient. By histology, 80 % of patients had secretory, hypersecretory or apocrine changes associated with their mammographically indeterminate Ca++.



Of the original 78 patients, 59 (76%) did not have immediate re-excision, and 47 (80% [47/59]) have been followed by radiology alone. Twelve patients (20% [12/59]) had subsequent diagnostic procedures within 6 to 36 months after initial diagnosis, all of them with benign results. Of the remaining cases, 6 died of unrelated disease, 5 had no subsequent follow up, and 8 patients (10%) had immediate re-excision since the initial mammographic impression did not correlate with the initial biopsy results ie.: diffuse clusters of Ca++ suspicious for DCIS on mammography versus benign partially sclerosed micropapilloma with associated Ca++.

Conclusions: Our data suggests that if a small BPL (≤1.3cm) is completely removed by MB or NCB, only mammographic or US follow up is required. Re-excision is indicated only if the initial bx results do not correlate with the initial mammographic impression or if interval changes are detected.

181 Hsulf-1 Expression Is an Independent Predictor of Poor Outcome in Breast Carcinomas

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Background: Heparan sulfate (HS) proteoglycans are important modulators of growth factor activity. The Hsulf-1 gene encodes for a HS modifying sulfatase. This enzyme desulfates HS glucosamine in its 6-O position and thus removes a sulfate group important for HS activity. Down-regulation of Hsulf-1 has been reported in carcinomas of the ovary, liver, and the head-and-neck area.
Design: Slides from a tissue array containing duplicate cores from 207 human breast carcinomas were subjected to chromogenic in situ hybridization with Hsulf-1 antisense mRNA and to Ki67 and ER immunolabeling. The slides were manually scored by two pathologists. Retrospective clinical follow-up information was available for up to 18.6 years.
Results: Of 204 interpretable cases, 50 (25%) showed absent or weak, 94 (46%) moderate, and 60 (29%) strong Hsulf-1 expression at the mRNA level. Univariate analysis revealed a significant correlation between Hsulf-1 expression and overall survival (p=0.014) and disease-free survival (p=0.0014). Hsulf-1 expression correlated significantly with modified Bloom-Richardson grade (p=0.0001), Ki67 labeling index (p<0.0001) and ER negativity (p<0.001), but not with tumor size or lymph node status. Multivariate analysis showed a marginally significant association between Hsulf-1 expression and overall survival (p=0.069) and a significant association between Hsulf-1 and disease-free survival (p=0.021).
Conclusions: Hsulf-1 is overexpressed in aggressive, rapidly proliferating and ER-negative breast carcinomas. Hsulf-1 is an independent marker for poor clinical outcome. The modification of carcinoma cell-associated or extracellular matrix HS polysaccharides by Hsulf-1 may contribute to loss of growth control and/or metastatic spread in breast carcinomas.

182 Ki-67 Index Is Diagnostically Useful in Distinguishing Benign Fibroepithelial Lesions in Young Females

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Background: The histologic distinction between fibroadenomas (FA) and benign phyllodes tumors (BPT) can be challenging in some cases. This is particularly difficult when examining tumors of young females. We set out to determine if the Ki-67 index of the glandular epithelial and stromal components of these lesions would help to distinguish between FA and BPT in young women.
Design: 21 FA and 24 BPT of females 25 years old or younger were retrieved from our surgical pathology files. Immunohistochemistry was performed on 4 μm sections from a representative lesional paraffin block using a monoclonal antibody against Ki-67 (clone 7B11, Zymed Lab. Inc.). The number of positive cells was manually counted per 1000 cells using an Olympus BX40 microscope (400X magnification). Positive stromal (Ki-67 S) and glandular epithelial (Ki-67 G) cells, each per 1000 stromal or glandular epithelial cells were counted in each case. The Spearman correlation coefficient was used to determine the degree of correlation between Ki-67 S, Ki-67 G, age, tumor size and tumor type. Stepwise multiple logistic regression was used to determine which parameters could be used to classify a tumor into its appropriate histologic category. The Wilcoxon rank sum test was used to compare levels of Ki-67 for the two histologic groups.
Results: The mean patient age was 19 (median 18) and 18 (median 18) in FA and BPT, respectively. The mean tumor size was 2.4 (median 2.0) and 3.2 (median 2.5) in FA and BPT, respectively. The mean Ki-67 S was 42 and 103 for FA and BPT, respectively. The mean Ki-67 G was 102 and 180 for FA and BPT, respectively. The Wilcoxon test showed significant differences between histologic tumor types for both Ki-67 S and Ki-67 G (P<0.0148, P<0.005, respectively) with Ki-67 index lower in the FA group. No differences were found for age and tumor size. No significant correlations between age or tumor size with either Ki-67 S or Ki-67 G were found. However, Ki-67 S and Ki-67 G were significantly correlated (r=0.70, P<0.0001).
Conclusions: Both Ki-67 S and Ki-67 G are good predictors of histologic tumor type. Logistic regression revealed that Ki-67 G was the better predictor (P<0.0048). The Ki-67 index is useful as a diagnostic adjunct in differentiating FA from BPT in this subgroup of patients where the histologic distinction can be particularly challenging.

183 alphaB-Crystallin: A Novel Marker for Metaplastic and Basal-Like Breast Cancers

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Background: We have recently demonstrated that the small heat shock protein alphaB-crystallin is a novel oncoprotein that is associated with poor clinical outcome in breast cancer. In gene expression profiling studies alphaB-crystallin has been shown to cluster with the basal-like subtype of breast cancer. These tumors have a "triple negative" immunoprofile (ER-/PR-/HER2-), express cytokeratin 5/6 and/or HER1/EGFR and comprise approximately 10-20% of breast cancer cases. Metaplastic breast cancer has a similar immunophenotype and has been postulated to belong to the basal-like subgroup. Interestingly, we have recently shown that alphaB-crystallin protein is expressed in basal-like tumors. Moreover, we have demonstrated that overexpression of alphaB-crystallin in non-tumorigenic mammary epithelial cells renders them tumorigenic in nude mice, resulting in mammary carcinomas with characteristics of metaplastic and basal-like tumors. We hypothesize that alphaB-crystallin contributes to the pathogenesis of metaplastic and basal-like tumors. Hence, we investigated the rates of alphaB-crystallin protein expression in metaplastic and basal-like breast tumors.
Design: Whole tissue sections from 50 breast cancer cases were identified for study. Using the criteria outlined above we identified 21 cases of basal-like breast cancer. Also included were 29 cases of metaplastic breast cancer. All immunohistochemical staining was performed using automated techniques. For detection of alphaB-crystallin we used a commercially available primary antibody (SPA-222; Stressgen Biotechnologies). Tumor cell staining was scored as follows: negative-0% staining; weak-1-29% staining; strong-30% or greater staining.
Results: alphaB-crystallin expression was observed in 80% of the cases tested (40/50). Metaplastic breast tumors displayed weak staining in 5/21 cases (24%) and strong staining in 11/21 cases (52%). Basal-like breast tumors displayed weak staining in 11/29 cases (38%) and strong staining in 13/29 cases (45%).
Conclusions: alphaB-crystallin is highly expressed in both metaplastic and basal-like breast cancers. These findings suggest that alphaB-crystallin may be a useful marker for metaplastic and basal-like tumors. Our results also provide additional evidence

that these tumors may share similar pathogenic mechanisms. Further studies to explore the biologic and clinical significance of alphaB-crystallin expression are warranted.

184 p16 (INK4a) Promoter Hypermethylation and Protein Expression in 140 Invasive Ductal NOS Carcinomas of the Breast

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Background: CpG island methylation has been associated with transcriptional silencing of tumor suppressor genes resulting in uncontrolled growth. The p16 tumor repressor gene can be inactivated by promoter region hypermethylation in many tumor types, including breast cancer. Relationship between p16 promoter hypermethylation, protein expression and other clinicopathological features was controversial. The aim of this study is to determine the frequency of promoter hypermethylation and expression of p16 in a selected group of 140 ductal NOS breast carcinomas, and their relation to other well known prognostic factors.

Design: Formalin-fixed, paraffin-embedded tissues from 140 patients with invasive NOS ductal carcinoma of the breast were stained for ki67, p16, c-erb-B2, p53 and hormonal receptors (ER and PR) by standard immunohistochemistry. The methylation status was investigated using methylation-specific PCR on DNA extracted from snap-frozen paired tumor.

Results: Hypermethylation of p16 promoter was found in 59 cases (42%). These tumors showed c-erb-B2 overexpression (p=0.027) and an high ki67 index (p=0.044) more frequently that those not methylated. None of these factors maintained their significance in multivariate analysis. No correlation were observed with size, histological grade, nodal status and ER and PR receptors. p16 expression was observed in 97 cases (69%), being the protein localization both nuclear and cytoplasmatic (69%) or nuclear alone (24%). Tumors with p16 expression were poorly differentiated (p=0.004), c-erb-B2 positive (p=0.004), expressed p53 (p=0.008) and lack ER (p=0.023) more frequently than tumors not immunoreactive for p16. Only c-erb-B2 showed significant correlation (OR=6, p=0.002) with p16 expression in multivariate analysis, while histological grade (p=0.066) and p53 expression (p=0.087) showed statistical trend. No significant correlation between p16 promoter hypermethylation and immunohistochemical detection of p16 protein was observed (p=0.13), although tumors with hypermethylation were more frequently p16 positive (75,4 %) that those not methylated (63,4%).

Conclusions: Hipermethylation of the p16 promoter region is a common event in ductal NOS breast carcinomas and it is associated with c-erb-B2 overexpression. Immunohistochemical detection of p16 is unrelated to promoter methylation status, being strongerly related to tumor characteristics of poor prognosis (c-erb-B2 overexpression and lack ER) than promoter methylation status.

185 Lymphomas of the Breast: A Review of 12 Years

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Background: Non-Hodgkin's Lymphomas (NHL) of the breast are rare and represent 0.04-0.50% of malignant lesions of the breast. Most cases have a B-cell phenotype.This series summarizes our experience over the last 12 years based on the WHO classification 2000. To the best of our knowledge this is the largest series of breast lymphomas till date.

Design: 50 cases of breast lymphomas were retrieved from the institute database and corresponding archival blocks and slides were retrieved spanning 1993- 2005. The pathology material was reviewed by a hematopathologist and breast pathologist. Immunohistochemistry and molecular studies were performed where relevant. Clinical information was obtained from patient charts.

Results: Age ranged from 34-95 years (mean 65.5 years). 45 cases had primary breast lymphomas, 8 cases- secondary breast involvement. Distribution: 30-right breast, 18-left breast, 2- bilateral. Primary lymphomas in descending order of frequency included Diffuse Large B-cell Lymphoma (DLBCL) 17/50, Follicular Lymphoma(FL) 10/50; 2/10- grade 2 follicular and diffuse pattern, 8/10 grade1(WHO classification). 8/50 cases: Marginal Zone lymphoma (MZL) with 1 case showing transformed DLBCL. 4/50 B-CLL, 2/50 Mantle Cell Lymphoma (MCL), 1/50 B cell -Acute Lymphoblastic Lymphoma/ Leukemia (ALL) and 1/50 Peripheral T cell Lymphoma (PTCL). Secondary breast involvement included 1 case each of DLBCL with bilateral axillary lymph node involvement, T-ALL with primary mediastinal involvement, Anaplastic Large cell lymphoma (ALCL) with overlying skin involvement and 2 cases of NK/T cell lymphoma with primary nasal cavity involvement. Bilateral breast disease was seen in cases of secondary involvement with DLBCL and T-ALL. At the time of diagnosis 17 cases had bone marrow involvement which included 2 cases of FL, 10 cases of DLBCL, 1 case each of MCL and B-CLL, both cases of ALL and one case of NK/T cell lymphoma. All cases were treated with lumpectomy or mastectomy followed by systemic chemotherapy regimen. Overall 5-year survival was 30%.

Conclusions: NHL of the breast is rare and most are B-cell phenotype, although our series had a few cases showing T-cell phenotype. In our series of 50 cases, DLBCL was the most common subtype (38%) followed by FL (20%), MZL (16%) and other subtypes accounting for 2-8% as described in the results. Rare subtypes such as ALL, ALCL, NK/T cell lymphoma and PTCL were also encountered in our series although the former 3 presented as secondary involvement in the breast. Histologic type and stage of disease at initial presentation impacted on overall survival.

186 Pathologic and Biologic Response to Neoadjuvant Anti-Estrogen (AE) Therapy in Patients with Ductal Carcinoma In Situ (DCIS)

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Background: Adjuvant AE therapy is commonly recommended for patients as treatment for DCIS. However, the effects of preoperative therapy of DCIS with AE have not been previously described. This study was undertaken to determine whether neaoadjuvant AE in DCIS results in detectable morphologic changes.

Design: Patients (n=14) with DCIS diagnosed on stereotactic core biopsy for mammographic calcifications enrolled in a trial of 3 months of neoadjuvant AE (tamoxifen for premenopausal patients and letrozole for postmenopausal patients) followed by definitive excision. Diagnostic mammograms and MRIs were obtained at baseline and immediately prior to surgery. Patients on hormone replacement therapy, with palpable masses or with clinical suspicion of invasion were excluded. Effects of AE were evaluated by 6 parameters on the pre- and post AE samples: morphologic changes, hormone receptor status by immunohistochemistry (IHC) for ER and PR, HER2 by IHC, proliferation rate by Ki67 IHC, apoptosis rate by active caspase 3 IHC, and intra- and periductal histiocyte density by CD68 IHC.

Results: The median age of the patients was 49 yr (41-65). Eight were pre- and 6 post-menopausal. Of the cohort, 13 were ER/PR+ and 3 were HER2+. Morphologically, 9 post-AE patients had multinucleated histiocytes and degenerated cells within DCIS and atypical ductal hyerplasia (ADH), and 1 patient showed only ADH with no residual DCIS. The ducts involved by DCIS were less distended. Ki67 expression decreased in these 9 patients by an average of 11%. Five patients lacked changes in morphology and Ki67 expression: 3 were HER2+, 2 used AE for < 6 weeks. Twelve of 13 patients had increased intraductal histiocytes by CD68. Minimal changes in the caspase 3 rate were observed (average change 2.2%), which did not correlate with changes in morphology, Ki67 expression or histiocyte density. One postmenopausal patient lost PR expression, others showed no significant change in ER or PR.

Conclusions: The effect of AE therapy on DCIS is associated with reduced proliferation and increased histiocyte reaction in a subset of patients, and does not appear to involve apoptosis by activated caspase 3. In this small data set, ER+ but HER2 overexpressed DCIS did not show any morphologic changes with neoadjuvant AE treatment. Preoperative AE treatment for DCIS is feasible, and further studies are warranted to determine whether women with DCIS responding to such therapy may safely avoid surgical intervention.

187 A Newly Proposed Semi-Automated Method of Grading Ductal Carcinoma In Situ of the Breast

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Background: Tumor size, margin status and grade are the most significant prognostic factors in determining the biologic behavior of ductal carcinoma in-situ (DCIS). Although architectural features of DCIS are of value, nuclear grade and necrosis have been shown to be more useful in predicting local recurrence and invasive transformation. The inclusion of necrosis and nuclear grade in the classification schemes of DCIS has demonstrated a general good agreement between pathologists in grading low and high grade DCIS. There was significant disagreement, however, with regards to the remaining intermediate group. Using our recently proposed (Kansas University-KU) grading system for invasive mammary carcinoma, we have demonstrated an agreement between the histologic grades and all of the histologic and prognostic markers studied and better prediction of patient survival than the Scarff-Bloom and Richardson (SBR) system.

Design: We adopted an automated grading system used for invasive carcinoma to grade DCIS. Tumors are graded based on nuclear pleomorphism and automated MIB-1 counts. Each component is given scores of 1 to3 and the combined score was grouped into grades of I to III. The nuclear component was given 3 scores as previously proposed and the MIB-1 count was scored 1 to 3 (1, <10%; 2, 10-20%; and 3, >20%). 162 DCIS tumors were studied including 39 Van Nuys (VN) grade I, 42 VN grade II, and 81 VN grade III cases. The VN and KU systems were compared with each other and correlated with tumor size, ER, PR, p53, Her-2, EGFR, Bcl-2, p27 and p21 status of each tumor.

Results: The KU, in comparison with the VN, system was correlated with a variety of histological and prognostic markers. The two grading systems demonstrated similar frequencies for the different histologic grades and a general agreement with each other for all of the biomarkers studied. Overall, there was concordance of 67% in terms of tumors being classified in the same grade by the two different systems (Kappa value = 0.49; P=0.001). The greatest difference between the two systems was observed for those tumors initially classified as VN Grade III with 10 % being "down-graded" to KU II and 12% of VN grade II being "down-graded" to KU I.

Conclusions: The KU system combining nuclear grade and MIB-1 count is a valid, reproducible grading system for invasive and in-situ breast carcinomas. It is automated, less subjective in assessing mitotic activity and necrosis and correlates with other prognostic markers.

188 Lobular Neoplasia on Core Needle Biopsy Does Not Require Excision

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Background: Lobular neoplasia (LN), encompassing atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) is often an incidental finding on core needle biopsies (CNB) performed either for radiologic densities, calcifications, or both. While generally viewed as risk factors for the development of breast carcinoma, the necessity for the lesions' excision is controversial.

Design: Review of our database yielded 98 CNB cases of pure LCIS, ALH or both. All cases containing LN accompanied by a second lesion (e.g. radial scar, atypical duct hyperplasia) requiring excision were excluded. In all cases the diagnoses were carefully correlated with radiologic findings. In cases performed to evaluate calcifications, the

specimen radiographs were viewed, and the histologic calcifications were correlated with the respective imaging targets in terms of size, number, and pattern. Radiographs of paraffin blocks and deeper sections were performed when necessary. In all cases the LN was an incidental finding. LN was diagnosed with strict criteria (uniform, small dyscohesive cells with bland nuclei and uniform chromatin). Cases of LN composed of or admixed with larger, more atypical cells, mitoses and/or single cell necrosis were excluded. Clinical and radiological followup data were gathered.

Results: 91 cases were performed for calcifications and 7 for masses. The patients ranged in age from 35 to 82 years. 45 patients had subsequent surgical excision and 53 were followed radiologically without surgical excision. Clinical and radiologic information on 42 of the 53 patients revealed that all were stable without disease with followup periods ranging from 6 months to 7 years. Of the 45 patients with a surgical excision, 42 (93%) had only LN on excision (with some ALH upgraded to LCIS). Of the remaining 3 cases on excision: one had residual LCIS and a separate 1 mm focus of infiltrating lobular carcinoma (clearly a finding incidental to imaging studies); one had DCIS admixed with LCIS (retrospective examination of this CNB by 2 blinded breast pathologists revealed focal larger, more atypical cells and focal mitoses); and one had atypical duct hyperplasia.

Conclusions: Lobular neoplasia diagnosed on core needle biopsy does not require excision provided that careful radiographic-pathologic correlation is performed (with specimen x-ray examination) and that cases with larger cells and proliferative activity (necrosis and or mitoses) are excluded. Close radiologic and clinical followup is safe and adequate.

189 Improved Detection of Breast Carcinoma Using Mammaglobin and Gross Cystic Disease Fluid Protein-15 (GCDFP-15) by Immunohistochemistry

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Background: Mammaglobin is a member of the secretoglobin gene family expressed almost exclusively in mammary epithelium. Previous reports have shown that it is a sensitive marker for breast carcinoma when compared to the more commonly used GCDFP-15. We performed an immunohistochemical (IHC) survey of mammaglobin expression in breast and non-breast tumors to assess utility in identifying breast carcinomas, particularly when used in combination with GCDFP-15.

Design: A series of 226 breast carcinomas and 275 non-breast carcinomas were tested for mammaglobin and GCDFP-15 by IHC using rabbit monoclonal antibody 31A5 and mouse antibody BRST-2 D6, respectively. Scoring was based on percentage of positive tumor cells: negative (0%), rare cells (<1%), focal (1-25%), variable (25-75%), uniform (>75%). Tumors were considered positive if >1% of cells showed expression. Sensitivity and specificity of detecting breast carcinoma were estimated.

Results: The sensitivities of mammaglobin and GCDFP-15 for breast carcinoma were 47.3% and 52.2%, respectively, with a combined sensitivity of 69.5% ($p<0.05$ compared to individual sensitivities). A significant percentage of breast carcinomas were mammaglobin pos./GCDFP-15 – (17.3%) and mammaglobin neg./GCDFP-15 + (22.1%). No mammaglobin expression was detected in carcinomas from stomach (16), colorectum (27), pancreas/biliary tract (27), lung (10), prostate (15), kidney (26), and thyroid (18), while some cases of ovary (10/59 17%), endometrium (3/10 30%), and skin adnexa (12/67 17.9%) were positive.

Conclusions: The sensitivity of mammaglobin in detecting breast carcinoma is comparable to GCDFP-15. Because of incomplete overlap of expression, evaluating these two antigens together significantly increases the ability to identify breast carcinoma. The specificity of mammaglobin is high in tumors other than from ovary, endometrium and skin adnexa. It therefore may be useful as part of a panel of IHC markers for identifying breast carcinoma in the context of carcinoma of unknown origin.

190 Do Apoptotic and Proliferative Markers Help Predict the Response to Systemic Therapy in Locally Advanced Breast Cancers?

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Background: Predicting the response to chemotherapy in breast cancer is a challenge, since most are treated with surgery before systemic therapy, making it impossible to measure objective tumor response. Neoadjuvant therapy (NT), allows monitoring of the response to specific agent with a better chance of reaching pathologic complete response (pCR). We analyzed tumor markers (including apoptotic and proliferative) to identify tumors with more likelihood to respond NT.

Design: Patients with locally advanced breast cancers (LABC) undergoing NT were identified. Biopsies and post treatment lumpectomy/mastectomy specimens were reviewed and prognostic markers recorded. Stains for Ki67, caspase-cleaved cytokeratin 18 (ccCK18; an apoptotic marker specific for epithelial cells), and p53 were performed when adequate tissue was available. Response to NT, measured with 4 Tesla MRI, was correlated to the tumor characteristics.

Results: 29 patients with stage I-III LABC were identified. Average age: 47.8 (range: 28-73). 11 had grade 3, 14 grade 2, 2 grade 1 carcinoma. In 2 patients the tumor was two small to allow grading. 20 patients had ductal, 7 lobular, and 2 had both ductal and lobular features. 2 patients had bilateral tumors. Information on the MRI determined tumor size was available in 24: average: 6.2cm (range: 2.5-11.4cm). All patients received NT. pCR was achieved in 6/29 (20.7%). Residual disease in 13/23 patients had lower Ki67 staining when compared to pretreatment biopsy. MRI in all patients studied (11/13) revealed decrease in size after 4 cycles of Adriamycin+cyclophophamide followed by Taxol.

	pCR (n:6)	Residual Disease (n:23)	Total (n:29)	
Tumor Size	7.3cm (n:6)	5.2cm (n:18)	6.2cm (n:24)	
Average Ki67+ cells	63% (n:5)	29.7% (n:20)	36.4% (n:25)	p=0.03
Average ccCK18+ cells	24.6% (n:5)	5.4% (n:18)	9.6% (n:23)	
ER +	1/6 (16.7%)	16/23 (69.6%)	17/29 (58.6%)	p=0.02
p53 +	2/5 (40%)	6/14 (42.9%)	8/19 (42.1%)	
HER2/neu +	1/6 (16.7%)	7/21 (30.4%)	8/27 (29.6%)	

not all patients had adequate tissue to perform all IHC testing. Two patients did not have HER2/neu data available

Conclusions: LABC that are ER negative and have higher Ki67+ cells are more likely to achieve pCR after NT. A decrease in proportion of Ki67+ cells in patients with residual disease after completion of NT correlates with decrease in tumor size at 4 Tesla research MRI.

191 D310 Mitochondrial Genome Instability in the Development of Breast Cancer

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Background: Mitochondria are cytoplasmic organelles that generate ATP through oxidative phosphorylation. A cell contains multiple mitochondria and thus many copies of mitochondrial DNA (mtDNA). This abundance of mtDNA allows for facilitated PCR amplification and analysis in tissue samples of limited cellularity where the quantity of nuclear DNA is insufficient for investigation. It has been suggested that mitochondrial DNA is often altered in the course of neoplastic development of duct carcinoma of the breast. The D310 poly-C mononucleotide repeat in the regulatory D-Loop region of the mitochondrial genome is a known hotspot for mutations in cancer cells. This study examined D310 alterations to determine the frequency of instability in this region in the development of breast cancer and the stage at which it occurs.

Design: Samples were collected from the files of the Department of Pathology at the University Health Network of patients diagnosed with duct carcinoma in situ (DCIS) and invasive breast cancer. Microdissection was used to isolate populations of tumour cells from formalin-fixed paraffin-embedded specimens. Several areas of each specimen were dissected, including normal breast epithelium, hyperplasia, DCIS, and invasive breast cancer. The D310 region of the extracted mitochondrial genome was investigated using PCR amplification and subsequent DNA fragment analysis on the CEQ 8000 Genetic Analysis System (Beckman & Coulter).

Results: Multiple samples (49) have been examined from thirteen cases of DCIS associated with invasive breast cancer. Initial fragment analysis revealed that 7/13 (54%) cases of DCIS or invasive breast cancer exhibited a change in D310 allelic length. Within this group, 6/7 (87%) demonstrated a progression in D310 genotype from regions of normal cellularity to hyperplasia, and then to DCIS and invasive breast cancer.

Conclusions: Our data suggest that alterations in length of the D310 mitochondrial microsatellite may be associated with tumour progression in duct carcinoma of the breast. Due to the abundance of mitochondrial DNA, this is a useful tool to study the relatationship between microdissected, histologically defined, small lesions.

192 A Nomogram To Predict for Malignant Diagnosis of Solid Lesions in a One-Stop Breast Unit

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Background: Preoperative diagnosis of breast lesions is mandatory in order to avoid unnecessary surgery. Diagnoses may be obtained either through core biopsies or breast fine needle aspiration (FNA) cytology. The only strong repeatedly demonstrated predictor for cancer diagnoses in this setting is the the Birad ACR classification. We aimed at evaluating which additional factors may guide the physician in the therapeutic/investigational decision.

Design: Patients referred for newly diagnosed breast abnormalities are seen in our one-stop diagnosis unit. They undergo multiple medical consultations and complementary investigations as necessary, with the aim to reach diagnosis as much as possible within the same day and to propose treatment plan as required. Solid lesions are first investigated by FNA, which is completed by core biopsy as indicated. FNA results are discussed to reach a consensus between the diverse physicians present (surgeons, radiologist, pathologist, oncologist). Data regarding patients and lesions characteristics, as well as results of explorations performed have been prospectively recorded. A multivariate analysis of factors predicting for a cancer diagnosis was performed and a nomogram was constructed using the R statistical package. It was validated by bootstrapping.

Results: 697 FNA were performed for solid lesions within a 1 yr period. Two thirds of them were ultrasound-guided. Median age of the patients was 56 (16-92). Median tumour size was 15 mm (2-20). 56% of the lesions appeared palpable. Cytological diagnosis was malignant in 369 (53%), suspicious in 59 (8.4%), benign in 247 (35%), and unsatisfactory in 22 (3%). Final diagnoses at 9 months median follow-up are: 423 carcinomas (61%), 262 benign (38%), 7 atypical hyperplasia (1.7%). 0% of 14 patients with Birad ACR2, 3.2% of 152 with ACR3, 43% of 171 with ACR4 and 97% of 353 with ACR5 lesions had breast carcinoma. In a multivariate analysis, ACR classification, age and palpability were independently associated with diagnosis of malignancy. The nomogram to predict malignancy based on ACR classification, age and palpability had excellent discrimination and calibration (area under the ROC curve = 0.95, $p< 0.001$).

Conclusions: Our study provides an original nomogram for prediction of the malignant nature of recently discovered solid breast lesions. Further data including epidemiological individual risk evaluation will be presented at the meeting.

193 Expression of Protein Arginine Methyl Transferases in Mammary Ductal Carcinoma

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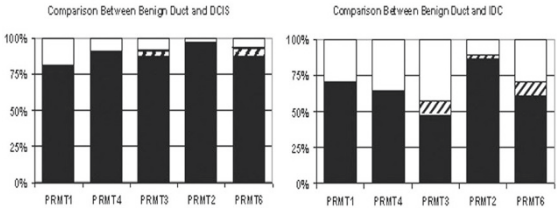
Background: Progression of ductal carcinoma of breast correlates with an altered gene expression profile. Protein arginine methyl transferase (PRMT) is a family of enzymes that catalyze the transfer of a methyl group to the nitrogen side chain of arginine. Methylation of specific nuclear proteins, such as steroid receptors including ER and AR, may result in modulation of signal transduction, nuclear transport or nucleic acid interactions. We sought to examine the extent of altered level of PRMT in human breast ductal carcinoma.

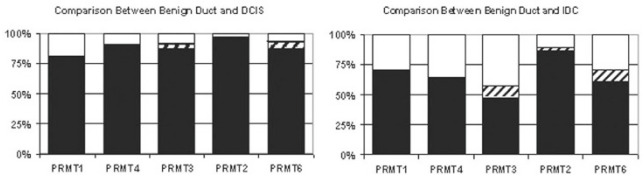
Design: 33 cases were selected from surgically removed invasive breast carcinomas. Formalin-fixed, paraffin-embedded tissue sections with cancerous and adjacent benign breast lobules were used for histology and *in situ* hybridization. The levels of cytoplasmic mRNA for PRMT1-4 and PRMT6 were semi-quantitatively assessed (Intensity score 0-3+).

Results: No significant change in mRNA levels of any of the PRMTs was identified in DCIS in the majority of cases (81-96%, Fig. 1, 2A, arrow head), when compared with terminal ductal lobular units (arrow). A small portion of these cases (4-19%) revealed reduced levels of PRMTs in DCIS. A greater percentage of invasive ductal carcinoma cases had reduced PRMT mRNA (10-43%, Fig. 2B, arrow head), compared with DCIS (Fig. 2B, arrow). Interestingly, a few cases exhibited elevated levels of PRMTs (5-10%).



Results are presentedas: No change (Solid bars), elevated (Striped bars) and reduced (Open bars) vs percentage of cases .

Conclusions: The levels of mRNA expression of PRMT are predominantly decreased in breast cancer progression. The reduction of PRMTs in invasive but not in situ carcinoma suggests that PRMTs may function as tumor suppressors and perhaps may protect against invasion.



194 Expression of α6β4 Integrin and Genes Upregulated by α6β4 Integrin Activation in Basal/Myoepithelial-Like Breast Cancer

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Background: Integrins are cell surface adhesion molecules with important signaling properties. Activation of α6β4 integrin, in particular, has been shown to play a role in tumor cell migration and invasion. Clustering of integrin receptors on the cell surface is an important mechanism for activating integrins. We evaluated changes in gene expression following cell surface α6β4 clustering in breast carcinoma cell line MDA-MB-231, known to have a high level of α6β4 integrin expression. We then evaluated immunohistochemical expression of selected genes found to be upregulated by α6β4 activation, together with expression of α6β4, in a subtype of breast carcinoma specimens previously identified by cDNA microarray analyses to have increased β4 integrin subunit gene expression, the basal/myoepithelial-like subgroup.

Design: MDA-MB-231 breast carcinoma cells were incubated with mouse monoclonal anti-β4 integrin or control anti-MHC1 on ice, followed by anti-mouse IgG for 2 hr at 37°C to crosslink the integrins and induce clustering. The experiment was performed in duplicate. Following RNA extraction, gene expression profiling studies were performed using GE CodeLink Bioarrays (55,000 genes). Gene expression data analysis was performed using GeneSpring software. For immunohistochemical staining, 59 basal/myoepithelial-like breast carcinomas, including 32 metaplastic carcinomas, were identified using previously reported criteria. Immunostaining for the β4 integrin subunit, EGFR and c-Met was performed on formalin-fixed, paraffin-embedded sections using automated immunohistochemical techniques, and staining was scored as negative (0), weak (1+), or strong (2+/3+).

Results: Of those genes found to be upregulated by α6β4 clustering, two (EGFR and c-Met) are receptors previously reported to co-precipitate with α6β4 in carcinoma cell lines. Increased gene expression of c-Met proto-oncogene was increased 3.3 fold, and EGFR was increasd 2.3 fold. Strong immunohistochemical expression of β4 integrin subunit, EGFR and c-Met was observed in 51%, 58% and 69% of the tumors, respectively.

Conclusions: α6β4 integrin activation in breast carcinoma cells increases expression of multiple genes, including EGFR and c-Met. Co-expression of α6β4 integrin, EGFR, and c-Met may be indicative of tumors with active α6β4 integrin signaling and increased invasive potential.

195 Detection of Monoclonal Lymphocytic Populations in Sentinel Lymph Nodes of Breast Carcinoma Patients

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Background: Breast carcinomas often contain tumor infiltrating T-and B-lymphocytes, however, it is unclear whether these infiltrates represent a tumor antigen-specific clonal immune response or they are merely a non-specific component of the inflammatory response. The sentinel lymph node (SLN) is the first LN draining primary breast tumors where priming of a specific lymphocytic response may take place. The goal of this study was to evaluate whether a tumor-specific response is detectable in SLN by analysis of T- and B-cell clonality.

Design: Forty five SLNs were stratified according to the size of metastatic tumor into 18 with macrometastases (>2 mm), 15 with micrometastases (<2 mm), and 12 negative nodes. Clonality was assessed by PCR of paraffinized tissues. T-cell receptor γ (TCR-γ) and immunoglobulin heavy chain (IgH) gene rearrangement analysis was used for T- and B-cell clonality, respectively. In addition, laser capture microdissection (LCM) was performed on CD20 and CD3 IHC stained SLNs sections to isolate cells of interest. PCR products were evaluated using high resolution microcapillary electrophoresis with the DNA 500 LabChip and Agilent Bioanalyzer.

Results: In the SLNs with macrometastases 7 of 18 cases (39%) demonstrated B-cell clonal rearrangements, whereas 4 (22%) were oligoclonal. In the SLNs with micrometastases a monoclonal population was detected in 5 of 15 cases (33%) and oligoclonal in 4 (27%). Negative lymph nodes showed significantly lower number of monoclonal rearrangements (2 of 12, 16%), while 3 of 12 (25%) were oligoclonal. The number of T-cell clonal rearrangements was lower than that of B-cells and did not differ significantly between the groups (17%, 7%, and 16%). Most of the cases with TCR-γ clonal rearrangements were positive for IgH rearrangement, suggesting that the clonal response involved both T- cell and B-cell populations. These findings were further confirmed by LCM with similar results. Moreover, using LCM analysis we were able to assess clonality separately in the lymphocytes infiltrating metastatic tumors as opposed to the lymphocytes away from the metastatic deposit. Finally, analysis of lymphocytic clonality was extended to lymphoid infiltrates in primary breast tumors.

Conclusions: Our study is the first to demonstrate a clonal B-lymphocytic response in SLNs positive for metastatic disease. Characterization and expansion of these clones may lead to the development of novel immunotherapies in the treatment of patients with breast carcinoma.

196 Breast Cancer with Features of Endocrine Ductal Carcinoma In Situ

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Background: Endocrine ductal carcinoma in situ (E-DCIS) is a rare entity. Tsang et al reported the largest series (N=34) of E-DCIS in 1996. However, E-DCIS still has not caught enough attention in routine surgical pathology practice. We here reported another 38 cases of breast cancer with E-DCIS features.

Design: 38 cases of breast cancer with features of E-DCIS were studied by using morphology, immunohistochemistry (IHC), and hsitochemical (HC) staining. The diagnostic criteria for E-DCIS were as follows: (1) Histologic patterns were compatible with the previous description of E-DCIS. (2) At least 50% of the DCIS cells were positive for 2 of the three neuroendocrine markers (chromogranin, synaptophysin and neuron-specific enolase).

Results: The clinicopathological features of E-DCIS were as following: (1) E-DCIS tended to occur in older woman. All the patients were over 58 years old in our group with the mean age of 69 years. The most common symptoms of the patients were either presenting a breast mass or nipple discharges. (2) E-DCIS had pure type (17 cases) and mixed type (21 cases). The mixed type was E-DCIS accompanied by an invasive component (In our group, 7 cases were with mucinous carcinoma and 14 cases with invasive ductal carcinoma). (3) Morphologically, E-DCIS showed expansile intraductal growth pattern. Intraductal papillomas were found in the vicinity of E-DCIS in 63% of our cases. (4) The tumor cells were polygonal, oval or spindle with abundant eosinophilic or granular cytoplasm. The nuclei showed mild to moderate pleomorphism. Intracellular or extracellular mucin could be shown by diastase-PAS or Alcian blue stainings. Some tumor cells had signet-ring appearance. (5) All 3 neuroendocrine markers were positive in E-DCIS lesions, which was futher confirmed with leu7 and CD56 stainings. (6) Pagetoid spread into the adjacent ducts was common in E-DCIS. Among the lesions showing expansile intraductal growth pattern, no myoepithelial cells were observed. These two features were helpful to distinguish E-DCIS from ductal hyperplasia.

Conclusions: E-DCIS represents a sub-group of low-grade DCIS, which has characteristic features in morphology and in IHC staining. Conventional microscopy permits the diagnosis in most cases. IHC and HC are helpful when microscopic diagnosis is uncertain. Further studies on clinical behaviour of E-DCIS are needed.

197 The Experience of Breast Cancer HER-2/neu Testing by Fluorescence In-Situ Hybridization in a Community Hospital: Advantages of Community Testing over Central Testing

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Background: Status of HER-2/neu oncoprotein offers critically important prognostic and predictive information for the management of breast cancer. Such importance was recently expanded from the previously recognized setting of metastatic disease to the management of localized disease. Therefore, accurate assessment of HER-2/neu status is now even more critical. Genetic testing by fluorescence in situ hybridization (FISH) has been shown to be a reliable and accurate method for tissue-based assessment of HER-2/neu. However, studies from cooperative oncology group trials warn that tests performed in community hospital laboratories were consistently less reliable than central laboratories. We recently validated and introduced HER-2/neu testing by FISH at our institution and wished to examine this hypothesis after the first 200 tests.

Design: An algorithm for testing of HER-2/neu at our institution was establshed. Newly diagnosed invasive breast cancers initially undergo HER-2/neu protein testing by immunohistochemistry (IHC, HercepTest, DakoCytomation). Tumors with equivocal IHC results (weak 2+ signal, heterogeneous signal, indeterminate signal such as edge effect) will undergo second testing by FISH (PathVysion, Vysis). Tumor's parameters were recorded including tumor's Nottingham grade, IHC score and FISH score.
Results: A group of 30 breast cancers was used as a validation set prior to implemeting FISH testing. 100% correlation rate was achieved between our lab and the reference lab prior to implementing FISH procedure locally. After the first 200 tests were conducted, FISH testing failure rate averages at < 1%, substantially lower than the national average failure rate of 10%.
Conclusions: Local testing of HER-2/neu by FISH is not only possible but can be more successful than in central laboratories. Part of the success is due to (a) the more homogeneous tissue fixation and processing procedures in the local setting compared to the central setting where specimens are received from a very large pool of histology laboratories, and (b) the necessary FISH testing and interpretation experience of our pathologists prior to implementing the test locally. We believe it is crucial to any pathology laboratory who wishes to impelment HER-2/neu testing by FISH to undergo an extensive validation process including at least 20 cases of borderline FISH results, before they proceed with clinical implementation of the test at their institutions.

Cardiovascular

198 Effect of Age on Aortic Intima Thickness and Intimal Soluble Elastin Fragments (sELAF) in Children and Young Adults in a Japanese Population

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Background: Intimal thickening in arterial wall begins in childhood. We have established a monoclonal antibody (HASG61-1) against sELAF using hybridoma technology. This antibody detects sELAF including tropoelastin, but not mature elastin. The purpose of this study was to investigate the relationship between intimal thickening (IT) and aging, and between IT and intimal sELAF in Japanese children and young adults.
Design: We performed quantitative pathologic analysis in human thoracic-abdominal aortic specimens of 104 autopsies (63 boys, age range, 0-18 years) using a computed digitized measuring system, and sELAF using immunohistochemistry.
Results: No atherosclerotic lesion formation was found in any of the specimens. Entire arterial wall thickness (EAWT) correlated with age, height, body weight (BW), and body mass index (BMI) [EAWT (μm) = 606.3+ 16.2× age (year), $R^2 = 0.262$, $p<0.0001$; 417.7+ 2.7 × height (cm), $R^2= 0.254$, $p<0.0001$; 581.8+ 6.5× BW (kg), $R^2 = 0.245$, $p<0.0001$; 533.5+ 12.1× BMI (kg/m^2), $R^2 = 0.051$, respectively]. Furthermore, arterial thickness of intima and media correlated with age [intimal thickness (μm) = 71.2+ 3.2× Age (year), $R^2 =0.99$, $p=0.0011$; medial thickness (μm) = 534.3+ 12.9× Age (year), $R^2 =0.237$, $p<0.0001$, respectively]. Meanwhile, intima/media ratio which is one of markers of atherosclerosis did not change through aging in the normal aorta. By immunohistochemistry, the sELAF were detected densely in the intima and weakly in the media of aorta. However, intimal sELAF did not change with aging.
Conclusions: Intimal thickening of aorta and sELAF detected in the intima occurs in early childhood and progresses with aging. These aortic wall changes are not associated with atherosclerosis but the arterial growth itself.

199 Expression of Soluble Elastin Fragments in Normal Human Aorta and Atherosclerotic Lesions

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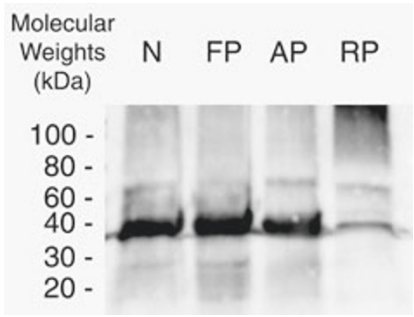
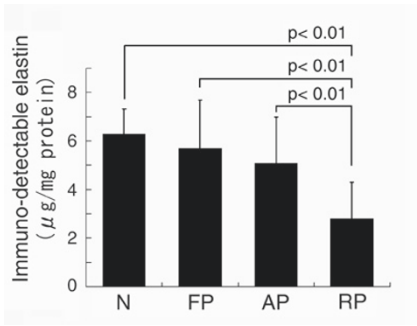
Background: Elastin is a major extracellular matrix component of arterial wall.
Design: Our aim is to assess the characteristics and roles of immuno-detectable elastin in normal aorta and fibrous, atheromatous and ruptured plaques (FP, AP and RP, respectively), using monoclonal antibodies against solubilized elastin fragments. We examined 172 human aortic specimens from 9 autopsies.
Results: Western blot analysis of the extracts of normal aorta and these plaques revealed several bands from 20 to 65 kDa: the 65 kDa band was tropoelastin. Decreased components in RP were elastin fragments ranging from 35 to 45 kDa. By immunohistochemistry, immuno-detectable elastin was abundantly distributed in the intima of normal aorta, but decreased in the core of AP and RP. The concentrations in RP were significantly lower than those in normal aorta, FP and AP. Elastin mRNA/GAPDH mRNA in FP and AP was significantly higher than that in normal aorta, respectively. Stepwise multivariable analysis showed that the concentrations of immuno-detectable elastin were significantly lower in the plaques having the rupture or large lipid pool than in those without these features.
Conclusions: These results indicate that, in the core area of AP and RP, loss of elastin fragments and negative elastogenesis may predispose in the plaque rupture.





200 Endomyocardial Biopsy To Validate Three-Dimensional Electroanatomic Voltage Mapping as a New Diagnostic Tool To Differentiate Right Ventricular Outflow Tract Tachycardia from Arrhythmogenic Right Ventricular Cardiomyopathy

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Background: Differential diagnosis between idiopathic right ventricular outflow tract (RVOT) tachycardia (VT) and segmental form of arrhythmogenic right ventricular cardiomyopathy (ARVC) is challenging in the clinical setting.
Design: By a new invasive electrophysiologic technique, ie three-D electroanatomic voltage mapping, the 3-D geometry of the RV depicting the peak-to-peak amplitude of the bipolar electrograms recorded at multiple endocardial sites is constructed. The technique allows to detect low-voltage areas that correspond to regions of myocardial atrophy, ie fibro-fatty replacement in ARVC patients. We assessed whether three-D electroanatomic voltage mapping can differentiate between idiopathic RVOT VT and ARVC by comparing the results with endomyocardial biopsy (EMB). .
Results: 24 consecutive patients (13 M and 11 F, mean age 34±11 years) with recurrent VT from the RVOT, and without echocardiographic evidence of RV dilatation/dysfunction were investigated. Activation mapping showed that all VTs arose from the RVOT. Voltage mapping was normal (>1.5 mV throughout the RV) in 17 of 24 patients (70%, Group A) and abnormal in the remaining 7 patients (30%, Group B), showing 2±1.4 areas with bipolar electrogram amplitude <0.5 mV (electroanatomic scar). Two patients from Group B had abnormalities limited to the RVOT, whereas in the other 5 the disease also involved the anterior (4), the inferobasal (3), and the apical (2) regions. At EMB, 6/7 patients from Group B (86%) versus 0/17 patients from Group A ($p= 0.001$) presented a diagnostic amount of myocardial atrophy (<45% of EMB section area) plus fibro-fatty tissue replacement, thus showing a strong correlation with RV electroanatomic scars. Catheter ablation successfully eliminated VT in 13 of 15 patients. During a follow-up of 26±9 months, 3 out of 7 patients (43%) from Group B received an ICD due to syncope or cardiac arrest, whereas all patients from Group A had an uneventful outcome ($p=0.02$).
Conclusions: Abnormal voltage map were identified in nearly 30% of patients with RVOT VT and no RV dilatation/dysfunction. Electroanatomic scar(s) correlated with myocardial atrophy and fibrofatty replacement at EMB as to confirm an underlying segmental form of ARVC at risk of life-threatening events

201 A Postmortem Investigation of Distal Coronary Microembolization in Acute Coronary Syndromes

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Background: Embolization of athero-thrombotic debris occurs in the setting of acute coronary artery thrombosis. Aim of our study was to evaluate the distal intramyocardial vessels in acute coronary syndromes in order to assess prevalence and features of coronary microembolization
Design: 60 pts who died due to an acute coronary syndrome (acute myocardial infarction-AMI- or sudden coronary death-SCD) with documented coronary artery thrombosis were evaluated; they consisted of 10 AMI pts who had thrombolysis (*Group A*, 5M-5F, mean age 68,5±14), 20 pts who underwent primary percutaneous coronary intervention (PCI) (*Group B*, 15M-5F, mean age 67±10), 18 AMI pts without coronary revascularization (*Group C*, 14M-4F, mean age 72±10) and 12 SCD pts (Group D, all M, mean age 33±10). Blocks of myocardium taken from both the perfusion territories of