

fibroblasts affect mammary epithelial cells using a coculture cell model. Benign (HB2) and malignant (MCF7) cells were stably transfected with a GFP expression plasmid, to enable subsequent differentiation from mammary fibroblasts in a coculture model, using flow cytometry. Cell proliferation was compared between epithelial and fibroblast cocultures when grown alone or in coculture using an endothelial cell line as a control. Malignant epithelial cell proliferation was enhanced by coculture with primary mammary fibroblasts, whereas benign epithelial cells showed dramatic proliferative inhibition. Two separate experiments were performed using each cell type and statistically significant *p* values were obtained: HB2 *p*=0.05, *p*<0.001; MCF7 *p*=0.001, *p*=0.019. The endothelial cocultures inhibited both mammary epithelial cell lines to a similar degree, suggesting that this was a mammary-specific event. Fibroblasts expressed multiple isoforms of ER beta by RT-PCR; additionally ER beta1 and beta2 were detected using Western blotting. Using a specific ER beta agonist (DPN) we noted changes in fibroblast proliferation and migration in response to wound healing and this is currently being extended to our coculture model using siRNA to knockdown ER beta. In conclusion, fibroblasts exert differential proliferative effects on mammary epithelial cells determined by their tumourigenicity and this may be mediated by ERbeta.

O-37 Fulvestrant versus exemestane following prior non-steroidal aromatase inhibitor therapy: efficacy and tolerability results from effect

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Background: Non-steroidal aromatase inhibitors (AIs; anastrozole and letrozole) are increasingly used as adjuvant and first-line advanced treatments for postmenopausal women with hormone receptor-positive (HR+) breast cancer. As many patients subsequently progress, it is important to identify agents with efficacy after non-steroidal AI failure.

Methods: EFACT is a randomised, double-blind, double-dummy, multicentre, Phase III trial comparing the efficacy and tolerability of fulvestrant (Faslodex®) vs exemestane (Aromasin®) in postmenopausal women with HR+ advanced breast cancer (ABC) progressing/recurrent after prior non-steroidal AI therapy. Fulvestrant loading-dose (LD) regimen was used: 500mg on Day 0, 250mg on Days 14, 28, and 250mg every 28±3 days thereafter. Exemestane 25mg was given once daily. Treatment continued until progression, death or withdrawal.

Results: 693 women were randomised to fulvestrant (*n*=351) or exemestane (*n*=342). ~60% of patients had received ≥2 prior endocrine therapies and ~60% had visceral involvement. Median time to progression was 3.7 months in both groups (hazard ratio: 0.963; 95% confidence intervals: 0.819, 1.133; *p*=0.6531). Objective response and clinical benefit rates (CBR) were also similar between groups. Median duration of response was 13.5 months vs 9.8 months for fulvestrant and exemestane, respectively. Fulvestrant and exemestane had good activity in patients with visceral disease (CBR 29.1% vs 27.2%, respectively). Both treatments were well-tolerated, with no significant differences in the incidence of pre-specified adverse events (weight gain, increased appetite, hot flushes, joint disorders, nausea/vomiting, diarrhoea, androgenic effects, injection-site reactions). Steady state plasma levels were reached within 1 month with the LD fulvestrant regimen.

Conclusions: Fulvestrant LD offers an effective and well-tolerated treatment option for postmenopausal women

with ABC (including those with visceral disease) who progress/recurrent on non-steroidal AI therapy.

O-38 Influence of a basal phenotype on the metastatic pattern of breast cancer

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Purpose: To assess whether basal phenotype (BP) influences metastatic pattern and survival in patients with metastatic breast cancer.

Materials and Methods: The BP status of a well-characterised series of consecutive primary operable breast cancers (1800 cases) was ascertained using basal CK5/6 and CK14. Follow-up data including time, site and pattern of distant metastasis and post-metastasis survival were available for 113 women with BP cancers and those were compared with 178 matching cases from women in the non-BP (NBP) group.

Results: Patients with the BP were more likely to present with intrapulmonary (25/48, [52%] BP vs. 15/64, [23%] NBP; *p*=0.0009) and/or brain metastases (20/113, [18%] BP vs. 3/178, [2%] NBP; *p*<0.0001). Patients with NBP were more likely to present with bone metastases in the absence of visceral disease (48/102, [47%] NBP vs. 14/62, [23%] BP; *p*=0.0017). There was no significant difference in the frequency of pleural or liver metastases between both groups. BP was also associated with a shorter median survival with metastatic disease (10.1 months vs. 25 months, *p*<0.001). Multivariate analysis including other established prognostic variables in metastatic breast cancer shows that BP is an independent poor prognostic factor.

Conclusion: Intrapulmonary and brain metastases are seen more frequently at metastatic presentation in BP breast cancer patients, and the BP is associated with a poorer survival after metastatic presentation. Thus assessment of basal CKs may provide valuable prognostic information that may affect patients' management.

O-39 Morphological and molecular evolutionary pathways of low and high grade breast cancers and their putative precursor lesions

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In this study, further support for our proposed route of pathogenesis concerning low grade and high grade breast cancers (LGBC and HGBC) and their precursor lesions was provided using immunophenotyping of tissue microarrays containing 790 lesions for putative tumour suppressor genes, cell cycle regulators, proliferation and differentiation markers. The putative precursor lesions were compared with their matching normal gland and invasive lesions.

Results: The epithelial cells in the flat epithelial atypia, Lobular neoplasia, ADH/ low grade DCIS and the intrinsic LGBC shared a common phenotype of CK19/18/8, ER-α, Bcl-2, and Cyclin D1 positivity. The ER-α/ER-β ratio and Cyclin D1 expression increased from precursor lesions to the invasive LGBC.

Conclusion: Our findings support the concept that FEA is the committed precursor cell lesions of LGBC/ILC. These may represent a family of precursor, in situ and invasive neoplastic lesions belonging to the luminal 'A' subclass of breast cancer. Our results suggest that the committed progenitor cells (PCs) for low grade breast neoplasia are CK19/18/8 positive and exhibit ER-α mediated CCND1 and BCL2 gene expression. Alternatively, breast cancer

(PCs) regardless of their original phenotype may acquire stochastic genetic and epigenetic hits that lead to the activation of separate histogenetic pathways for LGBC and HGBC, and that these events determine the phenotype of the pre-invasive and invasive lesions. Furthermore, these hits may be an early event in the progression of 'luminal A' tumours and once committed to this 'molecular pathway', progression to a 'high grade' (basal-like or HER2+) phenotype would be an unlikely.

O-40 An automated breast cancer grading demonstrator – PathScore

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Pathology analysis of tissue entails visual interpretation of complex microscopic images; this is liable to inter- and intra-observer variation. National External Quality Assessment Service (NEQAS) work has improved the concordance of grading from 0.3 Kappa to 0.6; however, the performance still varies. There is a need for a new approach.

The PathScore project has developed automated computer analysis for grading breast cancer using the Elston and Ellis grading scheme. The evaluation dataset consisted of 47 samples from the NEQAS assessed by 733 pathologists. Diagnostic features; gland (acinus) formation, nuclear atypia/pleomorphism, mitotic frequency and overall grade were scored automatically by PathScore. The majority view of all pathologists was used for this evaluation. The grade allocated by pathologists was usually not unanimously agreed, the level of agreement varied widely. Evaluation showed that PathScore's performance was similar to that of the human pathologists. Overall grade agreement between the Pathologists and PathScore was good, although there was some tendency for PathScore to overestimate the severity of Nuclear Pleomorphism. The observed agreement (68.09) is twice as high as could have been observed by chance. The weighted kappa of 0.591 is statistically very highly significant and there is no evidence of any significant disagreement over any category or its asymmetry. By coincidence the level of agreement on the final grade amongst the pathologists yielded an identical kappa value (kappa=0.59). PathScore provides the potential for enhanced objectivity and reproducibility offering a standardized reliable method for histological grading of breast cancer on routine clinical samples.

O-41 Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences

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The presence of vascular invasion (VI); encompassing both lymphovascular invasion (LVI) and blood vascular invasion (BVI), in breast cancer has been found to be a poor prognostic factor. It is not clear; however, which type of VI plays the major role in metastasis. The aims of this study were to use an endothelial subtype specific immunohistochemical approach to distinguish between LVI and BVI by comparing the differential expression of blood vascular (CD34 and CD31) and lymphatic markers (podoplanin/D2–40) to determine their prognostic role in a well characterized group of breast cancer patients with known long term follow up.

Sections from 177 consecutive paraffin-embedded archival specimens of primary invasive breast cancer were stained for expression of podoplanin, D2–40, CD31 and CD34. BVI and LVI were identified and results were correlated with clinicopathological criteria and patient survival.

VI was detected in 56/177 specimens (31.6%); 54 (96.5%) were LVI and 2 (3.5%) were BVI. The presence of LVI was significantly associated with the presence of LN metastasis, larger tumour size, development of distant metastasis, regional recurrence and shorter disease free interval (DFI) and overall survival (OS). In multivariate analysis, LVI was an independent prognostic factor for poor survival.

In conclusion, VI in breast cancer is predominantly of lymph vessels and is a powerful independent prognostic factor which is associated with risk of recurrence and death from the disease. The use of immunohistochemical staining with a lymphendothelial specific marker such as podoplanin/ D2–40 increases the accuracy of identification of patients with tumour associated LVI.

O-42 OSNA® for rapid molecular analysis of breast cancer lymph nodes: the Guildford experience

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Introduction: The OSNA® (One Step Nucleic Acid Amplification) system (Sysmex Corporation) has been developed to rapidly amplify CK19 mRNA from tissue lysates of lymph nodes (LNs), detecting metastatic tumour (>0.2 mm) in under 30 minutes. We are conducting a multicentre prospective study to determine concordance of OSNA analysis with multisection intensive haematoxylin & eosin (H&E) and immunohistochemical (IHC) examination in LNs of breast cancer (BC) patients. This abstract reports results of the initial technical phase from the Guildford Breast Unit.

Methods: Lymph nodes were removed using standard surgical techniques, defatted and cut into 4×1 or 2 mm slices. Alternate slices were snap frozen at –80° for subsequent OSNA analysis; remaining slices underwent H&E and IHC (CK19 and AE1/AE3) examination (0.25 mm multistep sections ×5 levels). Tissue lysates were prepared for OSNA according to standard procedure. OSNA was performed and results correlated with histopathology findings. Ethics approval was obtained prior to commencement of study.

Results: 45 axillary LNs (sentinel and non-sentinel) from 19 patients with BC were investigated by both OSNA and histopathological examination (figure 1).

N = 45		HISTOPATHOLOGICAL EXAMINATION (H&E AND IHC)				Total
		Positive		Negative		
		Macro Metastasis >2 mm	Micro Metastasis 0.2 – 2 mm	Isolated Tumour Cells* <0.2 mm	No Metastasis	
OSNA	Positive	8	3	2	1	14
	Negative	0	0	4	27	31
	Total	11		34		

Fig. 1. Results of LN analysis by Histology and OSNA ($p \leq 0.001$ chi squared); *considered 'histologically negative' (UICC TNM staging).

Overall concordance was 93.3%; sensitivity 100% and specificity 91.2%. Tissue allocation bias may explain some discordant cases; further investigation of these specimens is underway.