(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 from177 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 haemotoxylin & 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)				
		Positive		**Negative**		
		Macro Metastasis >2 mm	Micro Metastasis 0.2–2 mm	Isolated Tumour Cells* <0.2 mm	No Metastasis	Total
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 ($p \leqslant 0.001$ 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.

Conclusions: OSNA is a promising new molecular technique for rapid examination of SLNs in BC patients. The second phase of this study will investigate the efficiency of OSNA as an intra-operative diagnostic tool.

O-43 Global histone modifications in breast cancer tissue correlate with tumor phenotype, prognostic factors and patient outcome

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Background: Epigenetic changes in the form of global histone modification patterns have recently been shown to predict patient outcome in human prostatic carcinoma. However, the clinical significance of these modifications in breast cancer is unknown.
Methods: Seven specific antibodies were used to detect selected histone modifications in tissue microarrays of a large (n = 880) well-characterized series of human breast carcinomas using blinded semiquantitative scoring, in addition a set of well known markers in breast cancer.
Results: There is a highly significant correlation of histone modification status with tumor biological/morphological characteristics and clinical outcome. High levels of histone modifications were detected in luminal steroid receptor positive tumours, including lobular, mucinous and tubular carcinomas. However, significantly reduced levels of histone lysine acetylation (H3K9, H3K18, H4K12, H4K20), lysine methylation (H3K4, H4K16) and arginine methylation (H4R3) were observed in the poorer prognostic biological and morphological subtypes of breast cancer including basal and HER2-positive carcinomas, invasive duct carcinoma and medullary-type carcinoma. Low levels of these epigenetic marks were also associated with shorter disease free interval (DFI) and overall survival (OAS), particularly AcH3K18 that has an independent prognostic influence.
Conclusions: Our results show, for the first time, that global changes in specific histone modifications patterns may play an important role in breast cancer development and progression and their reduced expression is associated with poor prognosis and shorter survival.

O-44 Evaluation of estrogen and progesterone receptor, Her-2 and Topo IIα in primary breast cancer and metastatic axillary lymph nodes

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Background: Systemic treatment of breast cancer depends on different criteria, e.g. tumor size, grading, receptor status, Her-2/neu-Score. Usually these determinators are carried out using primary tumor tissue based on the assumption that the markers do not change during metastatic progression. We studied the concordance of estrogen (ER) and porgesterone receptor (PR), Her-2 and Topo IIα in primary breast cancer tissue and lymph node metastases.
Methods: We used paraffin-embedded tumor tissue from 118 patients with at least one ipsilateral metastatic lymph node. Immunhistochemistry (IHC) was used to analyze ER, PR, Her-2 and Topo IIα in primary tumor and lymph node. In Addition, Her-2 and Topo IIα amplification was evaluated by Fluorescence In Situ Hybridization (FISH) and Chromogenic In Situ Hybridization (CISH) in all samples with HER-2 Score 2+/3+ by IHC.
Results: Discordant results were seen in 2.56% (ER), 3.45% (PR), 3.42% (Her-2), 3.45% (Topo IIα) by IHC, respectively. However, using FISH and CISH, we found a complete concordance (100%) of the Topo IIα and HER-2 gene status between the primary tumor and the corresponding axillary lymph node. Comparing FISH and CISH, our results show a higher sensitivity with CISH detecting amplification of Topo IIα, whereas there was no difference in the detection of HER-2.
Conclusions: High concordance (approximately 96%) between primary tumor and metastatic lymph nodes of the examined biological markers was detected by Immunhistochemistry, and complete (100%) concordance using FISH and CISH. Nevertheless we recommend routine determination of Her-2 at metastatic lymph nodes, in order to treat all patients with Her-2 overexpression with trastuzumab. Regarding our results, HER-2 testing should be done with FISH, and Topo IIα should be detected by CISH, in order to obtain the highest sensitivity.

O-45 Reproducibility and interpretation of quantitative gene expression measurements in breast cancer biopsies

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Background and Objectives: Interpretation of genetic signatures on individual clinical specimens is needed to introduce quantitative gene expression measurements into clinical practice. In present study we measured mRNA expression of selected genes in homogenates of core biopsies to (i) evaluate the reproducibility of qPCR measurements in paired biopsies from the same tumour, (ii) to correlate measurements performed using qPCR and micro-array and (iii) to compare different ways of results representation.
Methods and Results: Repeatability of qPCR measurements in paired biopsies taken from the same tumour was studied for CCNB1 and MGB1 genes. Correlations between micro-array measurements and qPCR were studied for CCNB1, CDC2, NUSAP1, COLEC12, DCN, MMP2 and Ki67 genes. Relative coefficients of repeatability in qPCR were 2.2 and 15 fold for CCNB1 and MGB1 correspondingly. Exclusion of obvious outliners improved the coefficients of repeatability to 1.3 and 3.1 fold correspondingly. Positive correlations ($p < 0.001$) were observed between qPCR and micro-arrays for all studied genes except Ki67. Binary classification (ROC plots) and probability calculation (logistic regression) were compared to represent the outcomes of multi-gene quantitative measurements for interpretation.
Conclusions: (1) Paired biopsies taken from the same tumours may be used to validate quantitative gene expression measurements and possibility of their individual interpretation. (2) Representing results in the form of probability reflects the status of quantitative mRNA expression measurements better than presenting results as a discrete classification.

O-46 The prognosis of small breast cancers and selection for omission of adjuvant chemotherapy

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Aim: To recognise those cancers with excellent survival without adjuvant systemic therapy. Various studies have advanced criteria eg. all <10 mm (or) ⩽10 mm, LN neg, LVI neg, grade I.
Patients and methods: ONCOPOOL collected data from 16,893 operable (<5 cm) breast cancers aged 29–70 years, consecutively diagnosed in periods within 1990–99 at 13 European Breast centres. Women who received