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

S. El-Sheikh*, A.R. Green, E.A. Rakha, R.A. Ahmed, E.C. Pais, M.J. Grainge, G.R. Ball, J.F. Robertson, D.M. Heery, I.O. Ellis. *University of Nottingham, UK*

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

B. Ataseven*, D. Michler, B. Hoegel, M. Beer, W. Eiermann. *Red Cross Womens Hospital, Munich, Germany*

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

A. Larionov*, E. Murray, W.R. Miller, J.M. Dixon. *Edinburgh Breast Research Group, UK*

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

M. Sundquist*, M. Mitchell, R. Blamey, M. Blichert-Toft, L. Cataliotti, K. Holli, R. Jakesz, M. Kerin, I. Monypenny, M. van de Vijver, B. Hornmark-Stenstam, T. Pienkowski, On behalf of the ONCOPOOL Consortium

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

adjuvant systemic therapy have been withdrawn from this analysis.
Analyses: Breast cancer specific survival (BCS) by life table.
Results: Median follow-up 8.8 years (2.5–13.9). 2,499 cases were ⩽ did not receive adjuvant therapy.
Table I, of these 2499 cases 1051 were <10 mm

Diam		n	BCS survival 10 yr %
<10 mm	All	1051	94±1
<10 mm	LN neg, gr I	569	97±1
<10 mm	LN neg, gr III	142	83±3

To base selection of cancers with over 90% survival without adjuvant therapy on ⩽10 mm, LN neg, is insufficient; grade must be considered.
Table II, 2499 <20 mm analysed by the Nottingham Prognostic Index (NPI) NPI = Grade (I-III) + LN status (Neg, + 1–3, + >4) +size (cm ×0.2)

NPI	NPI Group	n	BCS Survival 10 yr %
	All <20 mm	2499	86±1
<2.4	Excellent (EPG)	1117	96±1
2.41–3.4	Good (GPG)	886	92±1
3.41+	Other Groups	496	All <90%

The addition of LVI to NPI made no significant difference to the results.
Conclusion: The highest sensitivity and specificity for selection of tumours with BCS over 90% at 10 years is by NPI, selecting 80% of all tumours ⩽20 mm (n = 2,003) and additionally recognising 264 >20 mm. This compares with ⩽10 mm, LN neg, grade I selecting only 1,051 cases.

O-47 Gene expression profile associated with docetaxel resistance in breast cancer cells

I. Brown*, S.D. Heys, A.C. Schofield. *University of Aberdeen, UK*

The mechanisms of resistance to docetaxel are poorly understood. The purpose of this study was to investigate the genetic pathways involved in docetaxel resistance using a unique model of docetaxel resistance, which we have developed in breast cancer cells.
We made two breast cancer cell lines, MCF-7 and MDA-MB-231, resistant by exposure to increasing docetaxel concentrations. The resultant sublines were able to withstand 1, 10 and 30 μM of docetaxel. Alterations of gene expression were determined using Affymetrix Genechip cDNA microarrays, and subsequently validated by RT-PCR and western analysis.
After selecting out gene changes that were common between both sets of sensitive cell lines and their resistant sublines (>2 fold), further normalisation and statistical filtering (ANOVA, assuming unequal variances, and the Benjamin-Hobbson false discovery rate applied as a multiple correction factor with a significance level of $p < 0.01$), we identified a 14 probe-set, encoding 10 genes (including p-glycoprotein), which were significantly associated with resistance to docetaxel. This probe set was interrogated for predictive value using Support Vector Machine algorithm (using Fisher's Exact test and Gaussian kernal function) and Principal Component Analysis on Conditions was applied to identify similar groups of gene expression between all the cell lines.
These changes, therefore, may represent common mechanisms of resistance in breast cancer cells, and may be able to predict response. In addition, this is the first description, using microarray analysis, to identify the genetic pathways involved in the evolution of acquired resistance to docetaxel in a cell line model.

O-48 Tolerability of zoledronic acid – first safety data from the AZURE Trial (BIG01/04)

R.E. Coleman, H. Thorpe*, D. Cameron, R. Bell, D. Dodwell, M. Keane, M. Gil, J. Cousins, R. Burkinshaw, on behalf of AZURE investigators. *Weston Park Hospital, Sheffield, UK*

The AZURE trial was designed to determine whether Zoledronic acid (Z) improves the disease-free and bone metastasis-free survival of women with stage II/III breast cancer. 3207 eligible patients received (neo)adjuvant chemotherapy (CT) and were randomised to no additional treatment or Z 4 mg iv every 3–4 weeks during CT, then every 3–6 months to 5 years. To correspond with timing of CT, serious (SAE) and non-serious adverse event (AE) data within 6 months of randomisation were compared.
939 SAE and 33859 AE have been reported to date. No significant differences in the numbers of patients with any SAE (324 [20%] CT, 373 [23%] CT+Z), or neutropaenic sepsis SAE (157 CT v 155 CT+Z respectively) were seen. CTC grade 3/4 AE occurred in 4.6% and 4.8% with CT and CT+Z respectively. The frequency of CT dose reductions (17% CT, 14% CT+Z) and median duration of CT (3.52 months CT, 3.48 months CT+Z) were similar, confirming that Z has no significant effect on CT delivery. 9 cases of osteonecrosis of the jaw have been confirmed to date (all reported cases).
This is the largest safety analysis of Z in patients without the confounding influence of metastatic disease and indicates that Z can be safely combined with adjuvant chemotherapy.

O-49 A phase III trial of Gemcitabine plus Docetaxel (GD) versus Capecitabine plus Docetaxel (CD) for patients (pt) with anthracycline-pretreated metastatic breast cancer

S. Chan*, R. Sharma, G. Romieu, T. Huober, T. Delozier, M. Tubiana-Hulin, A. Schneeweiss, A. Lluch, A. Llombart, A. du Bois, E. Carrasco, A. Thareau, P. Fumoleau, on behalf of trial investigators. *Nottingham City Hospital, UK*

Background: Patients (Pts) with anthracyclines pre-treated MBC frequently receive combination chemotherapy with a taxane and an antimetabolite such as gemcitabine or capecitabine. This trial compared the-(GD) combination with the (CD) combination, in this clinical setting. The primary objective of the trial was a comparison of the progression-free survival (PFS) difference between the two treatment groups, and the secondary objectives included overall response rate (ORR), time to treatment failure (TtTF), overall survival (OS), and toxicity assessments. In a previous analysis GD demonstrated similar efficacy to CD but with a better non-hematological toxicity profile [Chan et al, ASCO 2005]. This reports the final analysis of the results including OS.
Methodology: Pts with histologically/cytologically confirmed MBC, who had received an anthracycline-based regimen in the neoadjuvant/adjuvant/or first-line metastatic setting, were randomized to GD ($G = 1000\ mg/m^2$ d1, 8; $D = 75\ mg/m^2$ d1) or CD ($C = 2500\ mg/m^2$ daily d1 to 14; $D = 75\ mg/m^2$ d1) q21 days.
Results: Characteristics of the 305 included patients (GD = 153; CD = 152) were previously reported. A median of 6 cycles was delivered on both arms. CTC grade 3/4 hematologic toxicity was similar in both arms, except for grade 3/4 thrombocytopenia GD = 11%; CD = 3%; $p = 0.014$). The fact that blood test was performed at day 8 in the GD (pts received iv chemotherapy) but not the CD arm of the trial, may explain this difference. Nonhematologic toxicities were low in both arms, but