Results: The optimal signature contained 28 genes. There was a statistically significant correlation between actual versus predicted time to recurrence for blind data (rho = 0.975; $p < 0.0001$). A prospective Kaplan–Meier plot was generated which showed no significant difference to the actual Kaplan–Meier plot for this dataset ($p > 0.955$).

Discussion: For the first time gene expression signatures have been identified that predict actual time to event data rather than placing patients into arbitrary risk groups. Coupled with the ability to derive prospective Kaplan–Meier plots, this tool has the potential for assessing prognosis and determining treatment regimens on a case by case basis.

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O-34 DYNAMIC CONTRAST-ENHANCED MRI REVEALS CORE SIGNALLING PATHWAYS IN BREAST CANCER

S. Mehta [a], N.P. Hughes [b,d], F.M. Buffa [a], R.F. Adams [c], S.S. Gambhir [b,e], A.L. Harris [a]. [a] *Weatherall Institute of Molecular Medicine, Oxford, UK.* [b] *Institute of Biomedical Engineering, University of Oxford, Oxford, UK.* [c] *Oxford Breast Imaging Centre, Churchill Hospital, Oxford, UK.* [d] *Molecular Imaging Program, Stanford University, USA.* [e] *Department of Bioengineering, Stanford University, USA*

Dynamic contrast-enhanced MRI (DCE-MRI) is a widely used imaging modality for the management of breast cancer patients. At present, there is little understanding of how imaging patterns on DCE-MRI relate to the molecular pathways that drive tumour growth.

To address this issue, we performed a retrospective study of 65 patients with primary breast cancer, for whom pre-treatment DCE-MRI scans and formalin fixed paraffin embedded (FFPE) core biopsies were available. We used pharmacokinetic modelling of DCE-MRI to quantify the rate constant k_{ep} governing contrast agent washout from the tumour extravascular extracellular space. By computing the median k_{ep} over tumour volume an overall tumour leakiness score was derived. We extracted RNA from FFPE cores and measured gene expression using Affymetrix Human Plus 2.0 arrays. Following normalization and pre-processing, we used permutation tests to determine which genes were significantly correlated with median k_{ep}. Pathway analysis was performed using GeneCodis with the KEGG database.

Setting the False Discovery Rate to 5% resulted in 1258 genes that were significantly positively correlated with tumour leakiness including integrins B1 and A6, TGFBR1, HIF1 and 2A, SMAD4, HES1, JAG1. Interestingly, pathway analysis revealed that the p53 ($P < 0.004$), Wnt ($P < 0.004$) and Notch signalling pathways ($P < 0.006$), which are known to have important roles in angiogenesis, were all significantly associated with tumour leakiness.

These results illustrate how the combination of non-invasive imaging and gene expression profiling can reveal the molecular correlates of radiological features and provide insight into the mechanisms driving tumour growth and angiogenesis.

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O-35 SERPINB3, A BIOMARKER OF TAXANE BENEFIT IN BREAST CANCER

Elaina S.R. Collie-Duguid [a], Kathleen Sweeney [a], Keith Stewart [b], Iain Miller [b], Steve Heys [a]. [a] *Cancer Medicine, University of Aberdeen, UK.* [b] *Department of Pathology, University of Aberdeen, UK*

Background: Lysosomal cathepsin proteases function in a programmed cell death (LPCD) pathway. Although there is evidence for the importance of this pathway in cancer cell survival, it has not been exploited in anti-cancer therapeutics. Hsp70 and serpinB3 can block this pathway and promote cell survival. Furthermore, serpinB3 is associated with lack of response to chemotherapy. Cathepsin mediated cell death is observed in response to anthracyclines or taxanes, which are widely utilised in breast cancer treatment.

Methods: We evaluated serpinB3 and Hsp70 by immunohistochemistry in 255 surgically resected breast tumours from patients treated with either CVAP or CVAP and docetaxel prior to potentially curative resection. The study was performed with the approval of the regional research ethics committee.

Results: SerpinB3 and Hsp70 were significantly correlated with poor pathological response ($P = 0.014$ and $P < 0.0001$, respectively). SerpinB3 positivity is a poor prognostic factor ($P = 0.029$; mean survival 88.8 vs. 100.4 months) and this is independent in multivariate analysis ($P = 0.023$). Patients with serpinB3 positive tumours have poor survival if treated with anthracycline ($P = 0.026$) but not if they are also given a taxane ($P = 0.786$). Furthermore, only patients with serpinB3 positive tumours benefit from taxane treatment ($P = 0.008$).

Conclusions: SerpinB3 and Hsp70 are predictive biomarkers, potentially blocking breast tumour response to chemotherapy by preventing LPCD. SerpinB3 is prognostic and may prevent anthracycline-, but not taxane-, mediated cytotoxicity in breast tumours. Patients with serpinB3 negative tumours have a good prognosis when treated with anthracycline-based therapy alone. In contrast, patients with serpinB3 positive tumours benefit significantly from the addition of docetaxel.

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O-36 RECRUITMENT OF INSULIN RECEPTOR SUBSTRATE-1 BY ErbB3 IMPACTS ON IGF-IR SIGNALLING IN OESTROGEN RECEPTOR-POSITIVE BREAST CANCER CELLS

J.M. Knowlden [a], J.M.W. Gee [a], D. Barrow [b], J.F. Robertson [b], I.O. Ellis [b], R.I. Nicholson [a], I.R. Hutcheson [a]. [a] *Tenovus Centre for Cancer Research, Cardiff University, UK.* [b] *Professorial Unit of Surgery, Nottingham City Hospital, Nottingham, UK*

We have shown that insulin receptor substrate 1 (IRS-1) can associate with epidermal growth factor receptor (EGFR), with activation of EGFR promoting recruitment and phosphorylation of IRS-1 in an oestrogen receptor (ER)-positive tamoxifen resistant breast cancer (BC) cell line. In this study, we examined recruitment of IRS-1 by another erbB receptor family member, erbB3 in three ER-positive BC cell lines. Our studies revealed an interaction

between erbB3 and IRS-1 in MCF-7, T47-D and BT-474 cells with HRGβ1 treatment enhancing this recruitment and promoting IRS-1 phosphorylation at tyrosine (Y) 612, a specific PI3-K binding site. In addition, siRNA knockdown of IRS-1 greatly impaired HRGβ1 signalling via PI3-K/AKT in these cells. This novel interaction may have clinical relevance as immunohistochemical analysis of ER-positive BC patient samples revealed IRS-1 Y612 expression positively correlated with total erbB3, p-AKT and Ki67 expression. Importantly, we found that association of IRS-1 by erbB3 impaired IRS-1 recruitment by IGF-IR in both MCF-7 and T47D cells, whilst blockade of IGF-1R enhanced erbB3/IRS-1 interaction and sensitised both cell lines to HRGβ1. Consequently, knockdown of IRS-1 reduced HRGβ1 action and enhanced the effects of IGF-IR inhibition in these cells. In conclusion, these and previous findings suggest that IRS-1 can be recruited to IGF-1R, EGFR and erbB3 in ER-positive BC cells and this may provide an adaptive resistance mechanism when these receptors are targeted individually. Consequently co-targeting of IGF-IR and erbB receptors/IRS-1 may prove to be a more effective strategy for the treatment of ER-positive BC.

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O-37 SOX11 AND PSMD3 EXPRESSION IN HER2 POSITIVE BREAST CANCER

Y. Pang[a], G.R. Ball[b], E.A. Rakha[a], D.G. Powe[a], C. Caldas[c], I.O. Ellis[a], A.R. Green[a]. [a]*Division of Pathology, Nottingham University Hospitals, University of Nottingham, Nottingham, UK.* [b]*School of Biomedical and Natural Sciences, Nottingham Trent University, UK.* [c]*Cancer Research UK, Cambridge Research Institute, Cambridge, UK*

Human Epidermal Receptor 2 (HER2)+ have attracted attention as a poor prognostic class of breast cancer. However, HER2+ tumours appear to encompass biologically and clinically heterogeneous tumours.

In order to refine HER2+ breast cancer, we analysed over 48,000 gene transcripts in 132 invasive breast carcinomas using Artificial Neural Network analysis and identified high expression of two novel genes (SOX11, PSMD3) significantly associated HER2+ positivity. Using a large invasive breast carcinoma cohort ($n = 1{,}298$), prepared as tissue microarrays, we assessed these targets using immunohistochemistry and investigated associations with clinicopathological variables, patients' outcome and ability to refine HER2+ classification.

PSMD3 nuclear expression was observed in 219/942 (23%) of tumours and was significantly correlated to HER2 positivity ($p = 0.004$), tubule formation ($p = 0.047$) and NPI ($p = 0.007$). PSMD3 expression conferred a strong trend towards a longer breast cancer specific survival in the whole series ($p = 0.065$). SOX11 nuclei staining was observed in 96/869 (3.8%) tumours and was significantly associated with ER ($p = 0.006$) and PSMD3 nuclear ($p < 0.001$) positivity and ck14 negativity ($p = 0.018$) but not HER2. SOX11 expression did not predict patient clinical outcome in either the whole series or HER2+ tumours only.

This study confirms the biological and clinical heterogeneity of HER2+ tumours and the difficulties in translating global gene expression data into routine practice using immunohistochemistry. We have identified two novel genes associated with HER2+ tumours and further studies analysing the role of PSMD3 expression in this important subtype is warranted.

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O-38 DYSREGULATED CANCER-SPECIFIC MiRNAs IN THE CIRCULATION OF BREAST CANCER PATIENTS

N.A. Healy, H.M. Heneghan, A.J. Lowery, N. Miller, M.J. Kerin. *Department of Surgery, National University of Ireland, Galway, Ireland*

Introduction: Recent seminal findings from our institution indicate that systemic *miR-195* and *Let-7a* levels have potential as non-invasive breast cancer biomarkers. We aimed to validate these findings in an expanded cohort and to identify further miRNAs which augment the sensitivity and specificity of circulating miRNAs as diagnostic and prognostic markers for breast cancer.

Methods: The expression levels of nine miRNAs were evaluated in an expanded cohort of 265 breast cancer patients, 80 non-breast malignancies (colon, renal, prostate and melanoma) and 63 age-matched disease-free controls using RQ-PCR. Eleven additional miRNAs were evaluated as potential miRNA endogenous controls. Advanced QBase plus software and SPSS were used for biostatistical analysis of the data and correlation with clinicopathological variables.

Results: This study confirmed significantly deranged expression levels of systemic *miR-195* and *Let-7a* and two additional miRNAs in breast cancer patients compared to disease-free controls. Elevated *miR-195* was identified to be breast cancer-specific, with a sensitivity of 88% and a specificity of 91%. A combination of three circulating miRNAs, including *miR-195* and *Let-7a*, increased the discriminatory power of this test for breast cancer to 94%. Of the eleven candidate miRNAs selected for normalisation, two were identified to be stably expressed in a subset of the original cohort and thus are ideal endogenous controls for blood based miRNA studies.

Conclusion: This study highlights the presence and dysregulation of cancer-specific miRNAs in the circulation of breast cancer patients and illustrates the potential for this systemic miRNA signature to aid in the diagnosis and prognostication of this disease.

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O-39 HER2 POSITIVE EARLY BREAST CANCERS: WHAT PROPORTION ARE RECEIVING ADJUVANT TRASTUZUMAB THERAPY? A MULTICENTRE AUDIT

S. Marla[a], J. Cardale[b], D.J. Dodwell[b], A.I. Skene[c], O. Gojis[e], P. Abram[d], C. Palmieri[e], S.J. Cleator[f], A. Bowman[g], J.C. Doughty[a]. [a]*Western Infirmary, Glasgow and University of Glasgow, UK.* [b]*St. James's Institute of Oncology, Leeds, UK.* [c]*Royal Bournemouth Hospital, Bournemouth, UK.* [d]*Northern Ireland Cancer Centre, Belfast, UK.* [e]*Department of Oncology, Cancer Research UK Laboratories,*