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MEETING ABSTRACTS

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KEYNOTE LECTURE PRESENTATIONS

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Tissue banking
WF Symmans
Breast Cancer Research 2010, **12(Suppl 1):**L1 (doi: 10.1186/bcr2489)

Abstract not available at time of publication.

L2
Biomarkers for the diagnosis and prediction of therapeutic response in clinical breast cancer
J Bartlett
Breast Cancer Research 2010, **12(Suppl 1):**L2 (doi: 10.1186/bcr2490)

Abstract not available at time of publication.

L3
Recent advances in treatment of metastatic breast cancer
R Coleman
Breast Cancer Research 2010, **12(Suppl 1):**L3 (doi: 10.1186/bcr2491)

Abstract not available at time of publication.

SPEAKER PRESENTATIONS

O1
Male versus female breast cancer: a comparative study of 523 matched cases reveals differences behind similarity
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Retrospective studies on male breast cancer (MBC) have suffered from small numbers of cases available from any one centre; thus a significant problem in effectively studying this disease is accruing sufficiently large numbers to allow comparative analysis of biomarkers associated with response. Using a coordinated multicentre approach, we present the first large-scale study to address the relevance of the expression of hormone receptors in MBC and female breast cancer (FBC) using immunohistochemistry combined with a novel bioinformatics approach. Following ethical approval, 523 archival blocks (260 MBCs and 263 matched FBCs) were obtained retrospectively. Tissue microarrays were constructed and sections stained for ERα, ERβ1, ERβ2, ERβ5, total PR, PRA, PRB and AR and typed using CK5/6, CK14, CK18 and CK19 by immunohistochemistry. Following scoring, a range of ordination techniques were conducted on the datasets including hierarchical clustering and principal component analysis (PCA) to determine the differential nature of influences and interactions between MBC and FBC. Luminal A subgroup ($ER\alpha^+$ and/or PR^+, $HER2^-$) was the most common phenotype in both sexes. Luminal B ($ER\alpha^+$ and/or PR^+, $HER2^+$) was not seen in males, while basal-like tumours ($ER\alpha^-$, PR^-, $HER2^-$, $CK5/6^+$) were infrequent in both. Hierarchical clustering revealed common clusters between MBC and FBC including total PR-PRA-PRB and ERβ1/2 clusters. ERα occurred on distinct clusters between males and females. AR, ERβ1, ERβ2 and ERβ5 all existed on the same cluster but with a different substructure, particularly around the positioning of AR. ERα associated with this cluster in the male but not the female group. PCA confirmed that in both groups strong influences came from PR-PRA-PRB. In MBC strong influences additionally came from AR and ERβ1, ERβ2 and ERβ5, whereas in FBC strong influences came from ERα alone. Our data support the hypothesis that breast cancer is biologically different in male and females, which could have implications for therapy.

O2
Upregulation of ADAM proteases and HER ligands through a feedback loop mediates acquired resistance to trastuzumab in HER2-amplified breast cancer
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Breast Cancer Research 2010, **12(Suppl 1):**O2 (doi: 10.1186/bcr2493)

Introduction The response rarely sustains long among the responders for Herceptin (trastuzumab) monotherapy treatment. It is still poorly understood how Herceptin exerts its mechanism of action and how the acquired resistance to this drug occurs.
Materials and methods We used a multidisciplinary approach including fluorescence resonance energy transfer and biochemical methods to assess the effects of Herceptin on various signalling pathways and to determine the acquired resistance mechanisms of Herceptin in various HER2-positive breast cell lines and a BT474 xenograft model.
Results We have shown that Herceptin does not decrease HER2 phosphorylation despite the effect on HER2 receptor downregulation. HER2 phosphorylation is maintained by the activation of EGFR, HER3 and HER4 via their dimerisation with HER2 in breast cancer cells. The activation of EGFR, HER3 and HER4 is induced by HER ligand release, including heregulin and betacellulin. The release of HER ligands is mediated by ADAM proteases including ADAM17/TACE. Furthermore, we demonstrated that the feedback loop involving HER ligands and ADAM proteases is activated due to a decrease in PKB phosphorylation induced by Herceptin treatment. The feedback loop is also switched on when PKB phosphorylation is decreased by a PKB inhibitor. We have shown that the feedback loop activates the HER receptors and maintains HER2 phosphorylation in response to Herceptin. Herceptin in combination with a panHER inhibitor also caused a much greater tumour inhibition compared with Herceptin or panHER inhibitor alone in the xenograft model.
Conclusions Our data provide evidence that Herceptin as monotherapy may result in poor outcome for patients due to the escape mechanisms through a feedback loop involving the upregulation of ADAM proteases and HER ligands. We have provided a novel mechanism of acquired resistance to Herceptin in HER2-positive breast cancer and have resolved the inconsistencies in the literature regarding the effect of Herceptin on HER2 phosphorylation.

O3

New regulators of the BRCA1 response to genotoxic stress

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Breast Cancer Research 2010, **12(Suppl 1):**O3 (doi: 10.1186/bcr2494)

The breast and ovarian predisposition protein BRCA1 is a required component of the mammalian response to double-stranded DNA damage. Its conserved BRCT domains are required for BRCA1 accumulation to sites of repair, while the conserved N-terminal RING domain is able to catalyse the conjugation of ubiquitin and act as an E3 ubiquitin ligase. Disruption of either of these domains by missense mutation is associated with disease development.
The SUMO conjugation pathway has been implicated in DNA damage response in model organisms, and in *Caenorhabditis elegans* the Brac1 binding partner Bard1 associates with the SUMO E2 conjugating enzyme Ubc9. In mammalian cells, BRCA1 has been found to be associated with free SUMO-1 resulting in altered transcription.
We undertook to examine the potential influence of the SUMO pathway on BRCA1 response to genotoxic stress.
Using a range of biochemical and cell-biology techniques, we have shown that BRCA1 is modified by SUMO in response to genotoxic stress, and co-localises at sites of DNA damage with SUMO1, SUMO2/3 and the SUMO conjugating enzyme Ubc9. PIAS SUMO E3 ligases co-localise with and modulate SUMO modification of BRCA1, and are required for BRCA1 ubiquitin ligase activity in cells. *In vitro* SUMO modification of the BRCA1:BARD1 heterodimer greatly increases its ligase activity, identifying it as a SUMO regulated ubiquitin ligase. Further, PIAS SUMO ligases are required for complete accumulation of double-strand DNA damage repair proteins subsequent to RNF8 accrual, and for proficient double-strand break repair. Because the two features of BRCA1 activity regulated by the SUMO pathway, ubiquitin ligase activity and accumulation at sites of DNA damage, are also inhibited by some BRCA1 mutations that predispose to breast cancer and ovarian cancer, it seems highly likely that the SUMO pathway will be of relevance to cancer predisposition and development.

O4

DNA methylome of familial breast cancer identifies distinct profiles defined by mutation status

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It is now understood that epigenetic alterations occur frequently in sporadic breast carcinogenesis, but little is known about the epigenetic alterations associated with familial breast tumors. We performed genome-wide DNA methylation profiling on familial breast cancers (n = 33) to identify patterns of methylation specific to the different mutation groups (BRCA1, BRCA2 and BRCAx) or intrinsic subtypes of breast cancer (basal, luminal A, luminal B, HER2-amplified and normal-like). We used methylated DNA immunoprecipitation (meDIP) on Affymetrix promoter chips to interrogate methylation profiles across 25,500 distinct transcripts. Using a support vector machine classification algorithm, we demonstrated that genome-wide methylation profiles predicted tumor mutation status with estimated error rates of 19% (BRCA1), 31% (BRCA2) and 36% (BRCAx), but did not accurately predict the intrinsic subtypes defined by gene expression with error rates of 43% (basal) and 54% (luminal A). Furthermore, using unsupervised hierarchical clustering we identified a distinct subgroup of BRCAx tumors defined by methylation profiles. Finally, gene expression profiling and the SNP CGH array previously performed on the same samples allowed full integration of methylation, gene expression and copy number datasets. This integrated analysis revealed frequent hypermethylation of genes that also displayed loss of heterozygosity compared with the tumors that were diploid for that gene. We also observed frequent hypermethylation of genes that show copy number gains compared with diploid tumors providing a potential mechanism for expression dosage compensation. Together these data show that methylation profiles for familial breast cancers are defined by the mutation status and distinct from the intrinsic subtypes.

O5

Differentiation therapy: targeting breast cancer stem cells to reduce resistance to radiotherapy and chemotherapy

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Breast Cancer Research 2010, **12(Suppl 1):**O5 (doi: 10.1186/bcr2496)

Studies have shown that cancer stem-like cells (CSCs) from solid cancers are resistant to both radiotherapy and chemotherapy. We have shown that primary breast cancers (n = 8) and a breast cancer cell line (MCF7) enriched for breast cancer stem cells (BCSC) using mammosphere (MS) clonogenic culture can preferentially survive radiotherapy and chemotherapy treatment *in vitro*, showing ≥50% increase in MS survival compared with non-BCSC enriched cells. The BCSC enriched population, defined by the cell surface markers $ESA^+/CD44^+/CD24^{-/low}$, had reduced levels of DNA damage (measured by γH2AX) after 4 Gy irradiation or doxorubicin (1 μM) treatment. This suggests that the BCSC enriched population avoids or repairs the DNA damage significantly more than the whole population.
Differentiating agents have been used to re-sensitise breast cancers to endocrine treatment but effects on BCSC are unknown. All-trans-retinoic acid (ATRA), tricostatin A and vorinostat caused a dose-dependent decrease in the BCSC population using MS culture and FACS analysis after 72 hours of treatment in a monolayer. Vorinostat (100 nM) showed the greatest effect, with 80% reduction in the $ESA^+/CD44^+/CD24^{-/low}$ population and a 50% reduction in MS formation. Our data suggest that *in vitro* treatment with differentiating agents reduces the number of the BCSC within the MCF7 cell line.
Combination of ATRA (2 μM) or vorinostat with 6 Gy irradiation caused a significant reduction in MS survival showing a 30% and 70% decrease compared with an irradiated control. Similarly, in combination with paclitaxel (0.5 μM) ATRA and vorinostat caused a significant reduction in MS survival, showing 70% and 60% decrease compared with paclitaxel alone. In primary breast cancers (n = 3), combination of ATRA and 6 Gy irradiation significantly decreased MS formation by ≥25% respectively compared with irradiation alone.
These observations suggest that targeting BCSC with agents that eliminate or differentiate BCSC is a promising strategy to overcome resistance to radiotherapy and chemotherapy in the clinic.

O6

Transforming growth factor-beta co-receptor endoglin suppresses breast cancer invasion and metastasis

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Transforming growth factor-beta (TGFβ) signaling in cancer has been implicated in both growth suppression in early lesions as well as enhancing tumor cell invasion and metastasis. However, the cellular mechanisms that determine the signaling output in individual tumors are still largely unknown. In endothelial cells, TGFβ signaling is modulated by the TGFβ co-receptor endoglin (CD105). Here we demonstrate that endoglin is differentially expressed in invasive breast cancers and breast cancer cell lines, and is subject to epigenetic silencing by gene methylation. Downregulation of endoglin expression in nontumorigenic MCF10A cells leads to the formation of abnormal acini in 3D culture but does not promote cell migration or result in cell transformation. In contrast, in the presence of an activated oncogene, loss of endoglin in MCF10A cells leads to enhanced migration and invasion into a 3D matrix. Consistent with these data, ectopic expression of endoglin in the endoglin-negative MDA-MB-231 cell line blocks TGFβ-enhanced cell motility and invasion and reduces the ability of cells to successfully colonize the lung parenchyma in an *in vivo* metastasis model. Unlike endothelial cells, endoglin does not does not modulate canonical TGFβ signaling in breast cells but attenuates the cytoskeletal remodeling to impair cell migration and invasion. Importantly, lack of endoglin expression in clinical samples significantly correlates with *ENG* gene methylation and poor clinical outcome. Together these data identify endoglin as a key component suppressing the invasive activities of breast cancer cells.

POSTER PRESENTATIONS

P1

Activin B functions downstream of BRCA1 in stem cell maintenance

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Introduction The cancer stem cell hypothesis proposes that tumors contain a subset of stem or progenitor cells that are resistant to chemotherapy and have evaded the normally tight control of self-renewal. BRCA1 is known to play an essential role in cancer stem cell maintenance and differentiation. In fact, breast tissue from women with germline mutations in BRCA1 show regions of increased ALDH1 positivity, a marker of stem cells. Furthermore, knockdown of BRCA1 in normal tissue from reduction mammoplasty leads to increased ALDH1 and increased stem cell number as assessed by mammosphere formation.
Activin B is a secreted protein that is a member of the TGFβ family. It is a homodimer of inhibin βB that is expressed in various tissues and has numerous functions, including regulation of gonadal function, proliferation, metastasis, differentiation and stem cell maintenance.
Results Inhibin βB was identified as a transcriptional target of BRCA1 in SKBR7 cells on an Almac Breast-specific microarray. The target was analysed in several breast cell lines and shown to be repressed in basal-like, but not luminal, cell lines following BRCA1 knockdown or reconstitution. Chromatin immunoprecipitation analysis showed that BRCA1 is present on the INHBB promoter. Furthermore, loss of BRCA1 reduced the amount of secreted protein, which in turn correlated with reduced activation of the TGFβ pathway as shown by reduced phosphorylation of Smad2.
We have stably knocked down BRCA1 in MCF10A cells which, as expected, increased stem cell numbers in comparison with control cells as assessed by mammosphere formation and Aldefluor postivity. This defect can be rescued by addition of recombinant activin B or mimicked by inhibiting activin B activity with follistatin or SB-431542.
Conclusions We have identified inhibin βB as a novel transcriptional target of BRCA1 in basal-like cell lines. Preliminary phenotypic studies indicate that the homodimer of inhibin βB, activin B, functions downstream of BRCA1 in regulating stem cell numbers.

P2

FOXM1 is a transcriptional target of ERα and has a critical role in breast cancer endocrine sensitivity and resistance

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Breast Cancer Research 2010, **12(Suppl 1):**P2 (doi: 10.1186/bcr2499)

Previous data have shown that FOXM1 expression is elevated in breast cancer tissues and is strongly correlated with the expression pattern of ERα in breast cancer cells. The expression of ERα is a good prognostic factor in breast cancer, as about two-thirds of these ERα-positive patients respond to treatment with antiestrogens. However, approximately one-half of the patients that initially respond to hormonal therapy develop resistance. Since FOXM1 is critical for the progression of the cell cycle, we investigated the regulation of FOXM1 by ERα and its role in endocrine sensitivity and resistance in breast cancer cells. We firstly observed by quantitative RT-PCR a strong and significant positive correlation between ERα and FOXM1 mRNA expression in breast cancer patient samples. We showed that FOXM1 protein and mRNA expression was regulated by ER ligands. We also demonstrated that ectopic conditional expression of ERα, in the presence of estrogens, leads to induction of FOXM1 expression in ER-negative U2OS cells. Using reporter gene assays, we demonstrated that ERα activates FOXM1 transcription through an estrogen-response element site. The direct binding of ERα to the FOXM1 promoter was confirmed *in vitro* by mobility shift and DNA pull-down assays and *in vivo* by chromatin immunoprecipitation analysis. Importantly, silencing of FOXM1 by RNA interference abolishes the estrogen-mediated MCF-7 cell proliferation. Conversely, ectopic expression of a constitutively active FOXM1 can abrogate the cell cycle arrest mediated by the antiestrogen tamoxifen. Taken together, the results clearly demonstrate FOXM1 as a key mediator of the mitogenic functions of ERα and estrogen in breast cancer cells. Our findings that antiestrogens repress FOXM1 expression in endocrine-sensitive but not endocrine-resistant breast carcinoma cell lines and that ectopic expression of an active FOXM1 can abrogate the anti-proliferative effects of tamoxifen also suggest that deregulation of FOXM1 may also contribute to antiestrogen insensitivity.

P3

A novel and selective PDK1 inhibitor reduces breast cancer cell invasion and tumour growth

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Breast Cancer Research 2010, **12(Suppl 1):**P3 (doi: 10.1186/bcr2500)

Impairment of metastasis development is a critical target for cancer therapy. We recently reported that phospholipase Cγ1 (PLCγ1) is involved in regulation of motility and invasion of cancer cells and is required for metastasis development and progression. Experimental metastasis assays in nude mice revealed that inducible knockdown of PLCγ1 strongly inhibits development of MDA-MB-231-derived lung metastasis and reverts metastasis formation. In an effort to develop anti-metastatic drugs, different inositol phosphates compounds were tested to identify potential PLCγ1 inhibitors. We found that a synthetic derivative of inositol pentakisphosphate, Ins(1,3,4,5)P5, inhibits cell migration and 3D invasion in MDA-MB-231 and MDA-MB-435 human breast cancer cell lines and in TSA murine mammary adenocarcinoma cells and reduces calcium release upon EGF stimulation indicating a potential inhibition on PLCγ1 activity. Kinase profile assay, performed *in vitro* to test the potential inhibitory effect of the Ins(1,3,4,5)P5 synthetic derivative on different kinases showed a specific inhibition of the 3-phosphoinositide-dependent-protein kinase 1 (PDK1) with an IC_{50} of 26 nM. Knockdown of PDK1 using the small interfering RNA technology in breast cancer cell line MDA-MB-231 showed an impairment in cell migration and invasion and inhibition of EGF-induced calcium mobilisation. In addition, it has been recently shown that PDK1 is a critical determinant for resistance to tamoxifen anti-cancer drug. Our experiments show that combined treatment of the Ins(1,3,4,5)P5 synthetic derivative with tamoxifen, paclitaxel, and curcumin in MCF7 and MDA-MB-468 cells results in additive or more than additive effects, and therefore suggest that this novel PDK1 inhibitor can be potentially used in combination with other drugs to increase their anti-cancer activity.

P4

Regulation of mammary epithelial architecture by PTEN

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The PTEN tumour suppressor is a core component of the phosphoinositide 3-kinase (PI3K) signalling pathway. PTEN is mutated, deleted, or otherwise silenced in over one-third of breast cancers, with another one-third carrying other activating mutations in the core PI3K signalling pathway, most of these being activating mutations in the p110α catalytic subunit of PI3K itself. The best characterised tumour suppressor roles for PTEN are the suppression of cell growth, survival, proliferation and metabolic deregulation. However, we have found that in three-dimensional cultured mammary epithelial cells, knockdown of PTEN leads to the loss of cell polarity and tissue architecture. PTEN knockdown also causes a dramatic disruption of normal tight junction formation in adherent mammary epithelial cell cultures. Since the deregulation of cell polarity is becoming recognised as a potential driving force behind the formation of some tumours, we have been further studying the mechanisms by which PTEN controls mammary epithelial cell polarity.

P5

Genetic analysis of lobular carcinoma *in situ* and associated invasive lobular cancer

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Objectives This is a pilot study to assess the feasibility of performing SNP-loss of heterozygosity (LOH) on micro-dissected formalin-fixed paraffin-embedded

(FFPE) lobular carcinoma *in situ* (LCIS) tissue and to examine the genetic relationship between LCIS and associated invasive lobular carcinomas (ILC).
Introduction LCIS is a risk factor for the development of subsequent invasive breast carcinoma in either breast. Approximately 50 to 70% of these subsequent cancers are ILC. Not all LCIS progresses to invasive disease, and at present there are no biomarkers that predict which cases will develop ILC.
Methods LCIS and ILC samples were microdissected from FFPE tissue blocks. Genetic changes were studied using SNP-LOH (Goldengate Assay; Illumina) to assess CN-LOH and copy number changes. Copy number changes were confirmed using 32k BAC arrays. Ploidy was assessed using Feulgen staining.
Results SNP-LOH was successful in 31/35 samples. LOH events were more common in classical LCIS with associated ILC compared with pure LCIS. Commonest changes were on 16q and 1q. Other areas of LOH were more common in LCIS associated with ILC but did not reach statistical significance. Patterns of genetic change were maintained in ILC compared with the associated LCIS and 3/5 cases acquired additional genetic changes. Copy number changes were confirmed in three cases using BAC arrays. Concordance for copy number gain and loss was 50% and 75%, respectively. Concordance for copy neutral LOH was 100%. Ploidy studies on 20 cases revealed that all cases of classical LCIS were diploid whereas 5/9 pleomorphic LCIS/ILC samples were tetraploid or aneuploid.
Conclusions It is feasible to perform SNP-LOH on small amounts of micro-dissected FFPE LCIS tissue. The pattern of genetic changes confirms the findings of others that LCIS is a likely precursor of ILC. Further investigation of genetic changes in LCIS associated with ILC is expected to lead to the identification of biomarkers that predict for subsequent invasive transformation.

P6

Suppression of the NF-κB co-factor, Bcl3, delays the metastatic progression of breast cancer

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A large proportion of breast cancers overexpress the HER receptors, HER1 or HER2. Generally these patients have a poor prognosis, exhibit resistance to first-line anti-cancer drugs, and frequently develop metastatic disease – the most common cause of patient death. NF-κB transcription factors lie downstream of HER1/2 signalling pathways and are aberrantly activated in the majority of these breast tumours.
We have found that a constitutive deficiency in Bcl3 (an NF-κB co-factor that modifies NF-κB signalling) delayed HER2 (ErbB2) tumour onset and inhibited metastasis of mammary tumours in mice while growth of primary tumours was unaffected. In those Bcl3-null animals that did acquire metastases, the size of secondary tumours was significantly reduced compared with controls. Critically, Bcl3 deficiency did not affect normal mammary function other than having a transitory effect on apoptosis of epithelial cells in the post-lactational mammary gland. Therefore, unlike other NF-κB regulators, Bcl3 exhibited tumour-specific effects *in vivo*. The pro-metastatic properties of Bcl3 were confirmed in several human breast cancer cell lines exhibiting elevated HER2 and/or HER1 levels, including aggressive basal-like tumour cell types.
These observations are significant because they suggest it may be possible to target Bcl3 in established tumour cells to reduce metastatic behaviour, and furthermore that Bcl3's effects are not restricted to ERBB2-positive breast tumours, consistent with the observed increase in NF-κB activity seen in both ERBB1-positive and ERBB2-positive breast tumours.

P7

Identification of signalling pathways downstream of BRCA1 and p63

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Breast Cancer Research 2010, **12(Suppl 1):**P7 (doi: 10.1186/bcr2504)

BRCA1 was identified in 1994 as one of the genes predisposing to early-onset breast and ovarian cancer. It is currently estimated that 5 to 10% of all breast and ovarian cancer cases are inherited and the breast cancer susceptibility genes, BRCA1 and BRCA2, have been identified as being responsible for up to 21 to 40% of these cases. Although the exact function of BRCA1 remains to be defined, roles in DNA damage repair, cell cycle checkpoint control, transcriptional regulation and, more recently, ubiquitination have been inferred. p63 was identified as a positively regulated BRCA1 target gene through microarray analysis, and the functional significance of the BRCA1/p63 signalling axis was investigated.
Knockdown of BRCA1 and p63 leads to enhanced proliferation of breast cancer cell lines and increased stem cell numbers as assayed for by mammosphere culture and Aldefluor assay. Expression of BRCA1 or p63 in a background of low BRCA1 and p63 results in decreased cell proliferation. We therefore examined co-regulated targets of BRCA1 and p63 mediating growth control. S100A2, a tumour suppressor, is a known p63 target. Knockdown of BRCA1 and p63 leads to the loss of S100A2 expression. BRCA1 and p63 were found to be localised to the S100A2 promoter by chromatin immunoprecipitation assay. Loss of p63 resulted in recruitment of BRCA1 to the S100A2 promoter. In a p63 and BRCA1 null background, expression of S100A2 results in a reduction of cell proliferation. Conversely, loss of S100A2 in a BRCA1 and p63 expressing background leads to increased proliferation.
We have explored the regulation of signalling pathways by p63 and BRCA1 that are involved in growth control, differentiation and stem cell regulation. We will identify potential regulators of these pathways using microarray analysis to elucidate p63 and BRCA1 co-regulated targets.

P8

D133P53, directly transactivated by p53, prevents p53-mediated apoptosis without inhibiting p53-mediated cell cycle arrest

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We recently reported that the human *p53* gene encodes at least nine different p53 isoforms, two of which (p53β and Δ133p53) can modulate p53 transcriptional activity and apoptosis. In the present study, we aimed to investigate the regulation of Δ133p53 isoform expression and the physiological role of Δ133p53 in modulating p53 activities.
We report that in response to genotoxic stress, p53 transactivates directly the human *p53* internal promoter inducing Δ133p53 protein expression, which by differentially modulating p53 target gene expression prevents p53-mediated apoptosis without inhibiting cell cycle arrest. This indicates that Δ133p53 does not simply act at physiological level in a dominant-negative manner towards any p53 targets, but rather modulates p53 transcriptional activity in a promoter and stress-dependent manner. Hence, we have established a novel feedback pathway that modulates the p53 response, which might have an impact on p53 tumour suppressor activity. These observations may provide some explanations for the difficulties in many clinical studies of associating p53 status with cancer treatment and clinical outcome. Therefore, it would be interesting to determine whether Δ133p53 expression is associated with tumour markers, clinical outcome and cancer treatment in human cancers.

P9

Loss of CSMD1 disrupts mammary epithelial morphogenesis

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Breast Cancer Research 2010, **12(Suppl 1):**P9 (doi: 10.1186/bcr2506)

Introduction CUB and Sushi multiple domain protein 1 (*CSMD1*) is a candidate tumour suppressor gene of unknown function. *CSMD1* maps to chromosome 8p23, a region deleted in 50% of breast cancers (BC). We have examined the contribution of CSMD1 to the tumorigenic phenotype of mammary acini and evaluated its prognostic value in BC patients.
Materials and methods A shRNA CSMD1 MCF10A three-dimensional matrigel model was established. Moreover, functional assays were performed using shCSMD1 cell lines. CSMD1 was tested by immunohistochemistry in 275 BC samples.
Results Loss of CSMD1 in the MCF10A three-dimensional model resulted in an increased number of acini ($P = 0.001$), which are also larger in size (40%, $P = 0.02$) and misshapen relative to the control. Although expressing a high level of active caspase 3, shCSMD1 acini failed to form lumen.
Loss of CSMD1 expression caused a 56% ($P = 0.001$) increase in proliferation and a 44% ($P = 0.0006$) decrease in adhesion. shCSMD1 cells migrate much faster than control cells and showed 33% ($P < 0.001$) increase in invasion. These results were confirmed in two other cell lines.

Loss of CSMD1 expression was identified in 79/275 (28.7%) of BC cases, which was associated with high tumour grade (P = 0.003) and low overall survival (HR = 0.607, 95% CI = 0.4 to 0.91, P = 0.018). Moreover, CSMD1 is an independent predictor of overall survival (HR = 0.607, 95% CI = 0.4 to 0.91, P = 0.018) [1].

Conclusions Loss of CSMD1 affects cell adhesion, proliferation, migration and invasion, which lead to disruption of mammary duct formation. Loss of CSMD1 is associated with poor prognosis in BC, suggesting its use as a new prognostic biomarker.

Reference

1. Kamal M, Shaaban AM, Zhang L, Walker C, Gray S, Thakker N, Toomes C, Speirs V, Bell SM: **Loss of CSMD1 expression is associated with high tumour grade and poor survival in invasive ductal breast carcinoma.** *Breast Cancer Res Treat* 2009. [Epub ahead of print]

P10

Phosphoinositide 3-kinase class II beta is a novel target in breast cancer therapy

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The phosphoinositide 3-kinase (PI3K) family comprises eight mammalian isoforms grouped into three classes. Accumulating evidence suggests that the class II isoform PI3K-C2β may play a role in cancer development [1-3]. Indeed PI3K-C2β expression has been found increased in several cancers by gene expression profiling. Previously, we have identified a role for PI3K-C2β in cancer cell migration [3]. Here we show that PI3K-C2β is overexpressed in several human breast cancer cell lines as compared with normal breast cells. Downregulation of PI3K-C2β expression by shRNA inhibits oestradiol-dependent and heregulin-dependent growth of MCF-7 and T47D cells and soft-agar colony formation. Immunohistochemistry analysis of breast cancer tissues from 90 patients revealed that PI3K-C2β is not expressed in normal portions of breast tumour specimens (used as internal controls) and follicular breast tissues, whereas it is highly expressed in infiltrating ductal carcinoma breast cancer tissues. Interestingly, we found a highly positive significant (Spearman's rho test, P = 0.002) association between PI3K-C2β expression and the proliferative status (Ki67) of tissues analysed. In addition, we compared the expression levels of PI3K-C2β in 20 primary-metastasis pairs from breast cancer patients. We found that PI3K-C2β expression is significantly increased in lymph node metastasis with primary tumours (Wilcoxon–Mann–Whitney test, P = 0.001). Taken together these data suggest a correlation between PI3K-C2β expression and activation and breast cancer progression, and identify a novel molecular target.

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P11

MicroRNA-92 targets the 3′ untranslated region of ERβ1 mRNA and post-transcriptionally regulates its expression in breast cancer

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ERβ1 is often downregulated in cancer compared with normal cells, suggesting that it may function as a tumour suppressor and could play an important role in carcinogenesis. The expression of ERβ1 is regulated by multiple mechanisms such as methylation. Five splice variants of ERβ mRNA have been identified, ERβ1 to ERβ5. However, it is unclear whether and how the full-length version, ERβ1, is regulated post-transcriptionally.

MicroRNAs are a class of nonprotein coding small RNAs that regulate expression of genes at post-transcriptional levels. Using rapid amplification of 3′ complementary DNA ends (3′RACE), we have confirmed 3′ untranslated region (3′UTR) expression and sequences of ERβ1 mRNA in MCF-7 cells. Based on miRNA expression profiling of human breast cancer studies, we found that miR-92 is upregulated in malignant breast. *In silico* analysis using the *miRGen* database and RNA hybrid predicted that there are two putative miR-92 target sequences within the 3′UTR of human ERβ1 mRNA. Firstly, we profiled the expression of ERβ1 mRNA and miR-92 in breast cancer tissue and cell lines. miR-92 levels were higher in ERβ1-negative MDA-MB-453 cells than ERβ1-positive MCF-7 cells. We observed miR-92 upregulation in breast tumours while ERβ1 mRNA expression was decreased compared with matched adjacent normal tissues. We found a significant negative correlation between miR-92 and ERβ1 mRNA and protein in breast tumour (r = –0.5, P = 0.001 and r = –0.39, P = 0.037), respectively. Transfection of MCF-7 cells with anti-miR-92 increased endogenous ERβ1 mRNA and reduced cell proliferation. EGFP report experiment also confirms that the 3′UTR of ERβ1 carries the directly binding sites of miR-92. Finally, we showed that miR-92 expression is modulated by the ER ligands 17β-estradiol and tamoxifen in MCF-7 cells. These findings prove that ERβ1 expression is negatively regulated at a post-transcriptional level by miR-92. This miRNA could be considered a potential therapeutic target in breast cancer.

P12

Regulation of the apoptotic genes in breast cancer cells by the transcription factor CTCF

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CTCF is a highly conserved and ubiquitous transcription factor with versatile functions. We previously demonstrated that elevated protein levels of CTCF in breast cancer cells were associated with the specific anti-apoptotic function of CTCF. We used proteomics and microarray approaches to identify regulatory targets of CTCF specific for breast cancer cells. Among the CTCF identified targets were proteins involved in the control of apoptosis. A proapoptotic protein, Bax, negatively regulated by CTCF, was chosen for further investigation. Repression of the human *Bax* gene at the transcriptional level by CTCF in breast cancer cells was confirmed by real-time PCR. Two CTCF binding sites within the *Bax* promoter were identified by electrophoretic mobility shift assay and footprinting. In reporter assays, the *Bax*–luciferase reporter construct, containing CTCF-binding sites, was negatively regulated by CTCF. *In vivo*, CTCF occupied its binding sites in breast cancer cells and tissues, as confirmed by chromatin immunoprecipitation assay. Our findings suggest a possible mechanism of the specific CTCF anti-apoptotic function in breast cancer cells whereby CTCF is bound to the *Bax* promoter, resulting in repression of *Bax* and inhibition of apoptosis; depletion of CTCF leads to activation of *Bax* and apoptotic death. CTCF binding sites in the *Bax* promoter are unmethylated in all cells and tissues inspected. Therefore, specific CTCF interaction with the *Bax* promoter in breast cancer cells, and the functional outcome, may depend on a combination of epigenetic factors characteristic for these cells. Interestingly, CTCF appears to be a negative regulator of other proapoptotic genes (for example, Fas, Apaf-1, TP531NP1). Conversely, stimulating effects of CTCF on the anti-apoptotic genes (Bcl-2, Bag-3) have been observed. Taken together, these findings suggest that specific mechanisms have evolved in breast cancer cells to protect them from apoptosis; regulation of apoptotic genes by CTCF appears to be one of the resistance strategies.

P13

Fulvestrant-induced expression of erbB3 and erbB4 sensitizes ER-positive breast cancer cells to heregulins

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We have previously reported that induction of EGFR and erbB2 in response to antihormones may provide an early mechanism allowing breast cancer cells to evade the growth inhibitory action of such therapies and ultimately drive resistant growth. More recently, another member of the erbB receptor family,

erbB3, has been implicated in antihormone resistance in breast cancer. In the present study we have investigated whether induction of erbB3, and related family member erbB4, may provide an alternative resistance mechanism to antihormonal action in a panel of four ER-positive breast cancer cell lines. MCF-7, T47D, BT474 and MDAMB361 cell lines were exposed to fulvestrant (100 nM) for 7 days, and effects on erbB3/4 signalling and growth were assessed. Effects of the erbB3/4 ligand heregulin-β1 were also examined in the absence and presence of fulvestrant. Fulvestrant potently reduced ER expression and transcriptional activity and significantly inhibited growth in all four cell lines. However, alongside this inhibitory activity, fulvestrant also consistently induced protein expression and activity of erbB4 in the four cell lines and also promoted erbB3, erbB2 and EGFR protein expression and activity in MCF-7 and T47D cells. Consequently, fulvestrant treatment sensitised each cell line to the actions of heregulin-β1 with enhanced erbB3/4-driven signalling activity and significant increases in cell proliferation being observed when compared with untreated cells. Indeed, in T47D and MDAMB361, heregulin-β1 was converted from a ligand having negligible or suppressive growth activity into one that potently promoted cell proliferation. Consequently, fulvestrant-induced growth inhibition was completely overridden by heregulin-β1 in all four cell lines. In conclusion, these findings would suggest that although antihormones, such as fulvestrant, may have potent acute growth inhibitory activity in ER-positive breast cancer cells, their ability to induce and sensitize cells to growth factors, such as heregulins, may serve to reduce and ultimately limit their inhibitory activity.

P14

Risks of contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers

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Introduction Mutations in *BRCA1* and *BRCA2* confer high lifetime risks of breast cancer. Previous studies have suggested, following an initial diagnosis, the risk for a contralateral breast cancer is approximately 3% annually or up to 40% at 10 years. The results presented here are of contralateral breast cancer risk in *BRCA1/BRCA2* mutation carriers.
Methods Three hundred and seventy-four *BRCA1* mutation carriers and 346 *BRCA2* mutation carriers were followed up for up to 30 years following breast cancer diagnosis. The incidence of a contralateral breast cancer and the effect of tamoxifen use and oophorectomy on this were observed.
Results Follow-up over a 25-year to 30-year period shows a constant 2% annual risk of contralateral breast cancer in *BRCA1/BRCA2* mutation carriers. This risk is not affected by age at diagnosis of first breast cancer. Over the follow-up period, oophorectomy, if performed below the age of 45 years, led to a reduction in contralateral breast cancer risk of 40%. Tamoxifen use was shown to reduce the risk of a contralateral breast cancer in the first 6 years following initial diagnosis but no effect was seen after this period. Over the full follow-up period, tamoxifen use did not significantly reduce the risk.
Conclusions Women who carry a *BRCA1/BRCA2* mutation who have had breast cancer have a constant increased risk of a second contralateral breast tumour. Oophorectomy has a greater impact on reducing this risk than tamoxifen use.

P15

ADAMTS15 metalloproteinase inhibits breast cancer cell migration

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The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) family are a group of 19 extracellular, secreted proteases whose known functions include processing of procollagen molecules, cleavage of extracellular matrix proteoglycans and anti-angiogenesis. Our previous studies have shown that *ADAMTS15* is a novel predictor of good prognosis in breast cancer; patients whose tumours had high levels of *ADAMTS15* expression had an increased relapse-free survival compared with those with lower levels [1]. *ADAMTS15* has also emerged as a candidate cancer gene from cancer genome sequencing, and its tumour suppressive function has recently been documented in colorectal cancer [2].
Our study has focused on the cellular effects of overexpression in MCF7 and MDA-MB-231 breast cancer cell lines of full-length wild-type ADAMTS15 and an E-A mutant that lacks metalloproteinase activity. We have generated stable transfectants carrying either an inducible (lentivirus tet-off system) or constitutive vector system. The effects on cell adhesion, migration and proliferation have subsequently been analysed. Proliferation (MTT assay) and adhesion to various matrix components (including collagen, fibronectin and laminin) was not altered with the addition of ADAMTS15. However, ectopic expression (inducible and constitutive) of either full-length ADAMTS15 or the catalytically dead mutant significantly reduced migration in both cell lines. Wild-type ADAMTS15 also enhanced the aggregation of MCF7 cells. These data suggest that ADAMTS15 may exert tumour suppressive effects via modulation of the interactions of breast carcinoma cells with their environment independent of its metalloproteinase activity.

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P16

Macrophage-mediated breast cancer cell chemotaxis: the role of sphingosine kinase-1 activation

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Objective There is evidence to support the view that inflammatory processes are important in the development of local progression and metastases in patients with breast cancer. The sphingosine kinase-1/sphingosine-1-phosphate (SK1/S1P) pathway, which is a known mediator of inflammation, is critically implicated in breast cancer progression and chemotherapy resistance and is linked with poor prognosis. In this study we have investigated the implication of the SK1/S1P pathway in the interaction between tumour-associated macrophages and breast cancer cells.
Methods We have used modified Boyden chambers to perform macrophage-tumour cell co-culturing. Cytokine production and alterations in gene expression were measured by quantitative RT-PCR. Proteome profiler assays were used to identify secreted cytokines. Cell motility and chemotaxis were assayed in 96-well plates of Dunn chambers respectively using high-throughput video time-lapse scanning microscopy.
Results MDA-MB-231 breast cancer cells were pretreated with docetaxel and subsequently co-cultured with THP-1 macrophages. Macrophages exhibited increased chemotaxis towards apoptotic tumour cells (aTCs) or aTC conditioned media. Co-culturing with aTCs has transiently increased macrophage SK1 activity. Proteome profiling of media from macrophages revealed that aTCs induced an SK1-mediated secretion of IL-6 and siCAM-1. Interestingly, co-culturing with macrophages increased aTC chemoresistance. Incubation of untreated cancer cells with macrophages pretreated with conditioned media from aTCs induced an IL-6-mediated upregulation of cancer cell SK1 expression in cancer cells, which has lead to an increase in cancer cell motility and chemotaxis in gradients of macrophage conditioned media. These enhanced migratory phenotypes were reversed following treatment of cancer cells with SK1 siRNA.
Conclusions Our results suggest a novel IL-6/SK1-dependent mechanism of macrophage-induced breast cancer chemoresistance and metastasis.

P17

PLU-1/Jarid1B contributes to estrogen-induced cell proliferation in the normal mammary gland and in breast cancer, and is required for embryonic survival

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PLU-1/Jarid1B is a nuclear protein that is widely expressed in breast cancer, with higher levels being seen in ER^+ cancers. Expression in normal adult issues is largely restricted to testis and the differentiating mammary gland. Through the JmjC domain the protein can demethylate H3K4me3, which correlates with its function as a transcriptional repressor. PLU-1/Jarid1B contains a DNA binding domain, and can be recruited to DNA through binding to transcription factors. We now find that the protein interacts with the ERα receptor and contributes to

estrogen-induced survival of MCF-7 cells in *in vitro* culture and when grown as tumours in nude mice.
To investigate the function of Plu-1/Jarid1B *in vivo*, transgenic mice expressing defective Plu-1/Jarid1B have been developed. The systemic KO is an embryonic lethal with no homozygote embryos being detected at day 7.5. Another strain expressing the protein missing the ARID AT-rich DNA binding domain (which is also required for demethylase function) shows a mammary phenotype. In these ΔARID mice, the development of the mammary tree at puberty and early pregnancy is delayed, but the gland recovers by late pregnancy. The inhibition of development of terminal end buds at puberty, which is crucially dependent on ERα signalling, suggests an involvement of Plu-1/Jarid1B in this signalling that is impaired in the ΔARID mouse. Confirming this, levels of expression of downstream targets of ERα (progesterone receptor and Wnt4) are reduced in the ΔARID mouse. The development of spontaneous mammary tumours in the ΔARID mouse is delayed compared with wild-type mice, suggesting that Plu-1/Jarid1B contributes to tumour growth, and that this action is impaired when the ARID domain is deleted.
The data suggest that PLU-1/JARID1B is involved in estrogen-induced growth of normal and malignant mammary epithelial cells.

P18

C-terminal binding proteins play a critical nuclear function in the mitotic fidelity of breast cancer cells

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We are interested in finding novel therapeutic targets for breast cancer, particularly focusing on C-terminal binding proteins (CtBPs) as when they are inhibited, cells show an increased sensitivity to apoptotic stimuli. Using siRNA to inhibit CtBP expression, we have found that CtBPs are essential for the survival of breast cancer cells, and in particular those with a more aggressive p53-mutant phenotype. To direct our future studies into therapeutic strategies targeting CtBPs in breast cancer, we need to know more precisely how their loss results in death, and which of their functions are required for this prosurvival role.
Here we show that loss of CtBP function through siRNA treatment suppresses proliferation through a combination of p53-independent apoptosis, reduction in cell-cycle progression into mitosis, and aberrations in transit through mitosis itself. This third phenotype includes errors in mitotic chromosome segregation, activation of, but failure to sustain, the spindle assembly checkpoint, decreased expression of Aurora B, and a high rate of failure to complete cytokinesis. We showed that loss of CtBP in breast cancer cells with a functional p53 response pathway resulted in a marked upregulation of the p53 protein. Here p53 appears to be providing a protective role by arresting aberrant cells in G_1, thus preventing them from entering S-phase with incorrectly segregated DNA.
CtBPs are known to act in the nucleus as transcriptional co-repressors and in the cytoplasm as regulators of Golgi fission. Using a series of dominant negative CtBP mutants microinjected into either the cytoplasm or nucleus, we show that localisation of CtBPs to the nucleus is critical for its function in ensuring the correct division of breast cancer cells. This suggests that CtBPs function in maintaining mitotic fidelity, and thus in the continued proliferation and survival of breast cancer cells through their actions as a transcriptional co-repressor within the nucleus.

P19

RhoBTB2 in breast cancer

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Introduction Rho GTPases have multiple roles in cancer. We are working to characterise the novel Rho GTPase RhoBTB2/DBC2, which has been reported to be a tumour suppressor in breast cancer.
Materials and methods We used siRNA to mimic the loss of RhoBTB2 expression in breast cancer and then microarray analysis to identify the gene targets of RhoBTB2.
Results Screening identified the homeostatic chemokine CXCL14/BRAK as a target of RhoBTB2. CXCL14 is highly expressed by normal epithelial cells; however, its expression is downregulated in a wide range of carcinomas. We found that expression of both RhoBTB2 and the closely related RhoBTB1 gene are required for CXCL14 expression in epithelial cells. Loss of RhoBTB2 expression in cancer correlated with loss of CXCL14, and re-expression of RhoBTB2 in cancer cells restored CXCL14 expression.
Conclusions The high incidence of downregulation of RhoBTB2 (ca. 60%) and RhoBTB1 (ca. 50%) found across a wide range of carcinomas is sufficient to explain the observed frequency of downregulation of CXCL14. We propose that downregulation of RhoBTB1/2 represents the causative mechanism for altered CXCL14 expression in cancer cells. Previous work has shown that CXCL14 expression is lost from mammary epithelial cells in ductal carcinoma *in situ*, but is upregulated in the surrounding myoepithelial cells. This is suggestive of an autocrine to paracrine switch. In keeping with this, we find that exogenous CXCL14 disrupts the organisation of mammary cell acini grown in three-dimensional culture. Similar switching in CXCL14 production from epithelium to stroma has been reported in prostate and oral carcinoma. We are currently investigating the contribution of CXCL14 on invasive behaviour.

P20

Transcriptional regulation of cyclin-dependent kinase inhibitor 1A (P21) by the transcription factor AP-2γ

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Breast Cancer Research 2010, **12(Suppl 1):**P20 (doi: 10.1186/bcr2517)

Introduction AP-2 transcription factors constitute a family of sequence-specific DNA-binding proteins encoded by five highly homologous yet functionally distinct genes, AP-2α to AP-2ε. AP-2γ appears to play a major role in breast cancer, being expressed in a large proportion of primary tumours. In this study we have analysed in more detail the mechanism of transcriptional regulation of the p21/cyclin-dependent kinase inhibitor 1A (*p21/CDKN1A*) gene by AP-2γ.
Materials and methods Silencing of AP-2γ was carried out in MCF-7 cells using siRNA or doxycycline inducible shRNA. Chromatin immunoprecipitation (ChIP) assays were performed using specific antibodies against AP-2γ (H77), AP-2α, Myc, histone demethylase PLU1/JARID1B, histone H3 and trimethyl dimethyl and monomethyl histone H3 followed by quantitative PCR. Electrophoretic mobility shift assay (EMSA) competition assay and reporter assays were used to identify the AP-2 binding site.
Results Silencing of AP-2γ by either siRNA or inducible shRNA inhibits cell proliferation and results in upregulation of p21/CDKN1A expression with no induction of apoptosis. ChIP assays demonstrated binding of AP-2γ, PLU1/JARID1B and Myc to a region adjacent to the transcription start site of the *p21/CDKN1A* gene. Reduction in the binding of cMyc and PLU1/JARID1B and increased levels of histone H3 trimethyl-K4 were observed at the proximal region of *p21/CDKN1A* promoter after silencing of AP-2γ. Treatment of MCF-7 cells with the antimitotic drug vinblastine but not with hydroxyurea reduced the *CDKN1A* binding of AP-2γ, PLU-1/JARID1B and Myc. H3396 cells treated with the oestrogen receptor inhibitor Faslodex, which upregulates p21/*CDKN1A*, decreased AP-2γ binding but increased binding of AP-2α at the *p21/CDKN1A* promoter. EMSA competition assays and reporter assays showed that AP-2γ and AP-2α bind to a new site (GCC N3 GGG) at position –105 of the *p21/CDKN1A* promoter.
Conclusions The repression of the *p21/CDKN1A* gene by AP-2γ may contribute to the activation of proliferation associated with this transcription factor in breast cancer.

P21

Developmental protein HOXC11 cooperates with SRC-1 in breast cancer: an adaptive response to endocrine therapy

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The ability of a tumour to adapt and overcome targeted therapies has been recognised clinically for some time, but the molecular mechanisms driving this metamorphosis remain unclear. The steroid receptor coactivator protein, SRC-1, is a strong predictor of reduced disease-free survival in breast cancer patients. SRC-1 can also interact with nonsteroidal transcription factors, and defining these new transcriptional networks will uncover fresh strategies for managing endocrine resistance.
Here we employed a mass spectrometry-based screen to identify proteins that are specific to the endocrine-resistant phenotype. The developmental protein,

HOXC11, was identified and functionally validated as an interaction partner of SRC-1. We provide evidence that HOXC11 and SRC-1 cooperate to regulate expression of the calcium binding protein S100β in resistant breast cancer cells. Moreover, both nuclear HOXC11 and S100β were found to be strong predictors of poor disease-free survival in breast cancer patients (n = 560; hazard ratios = 5.79 and 5.82, respectively; P <0.0001). Elevated serum levels of S100β detected in patients also predicted reduced disease-free survival (n = 80; hazard ratio = 5.3; P = 0.004). This translational study identifies a biomolecular interaction network central to the adaptive response to endocrine therapy with clear clinical applications.

P22

Insulin-like growth factor binding protein-2 alters the sensitivity of breast cancer cells to chemotherapy

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Introduction Insulin-like growth factor binding protein-2 (IGFBP-2) is often elevated in breast tumours and the presence of IGFBP-2 has been shown to correlate with malignancy. Previously we have shown that IGFBP-2 reduces PTEN abundance [1] and thus helps to maintain the activity of the phosphoinositide 3-kinase signalling cascade, a key mitogenic and survival pathway.
Objective We therefore investigated whether IGFBP-2 could act as a survival factor for breast cancer cell lines. Using MCF7 and T47D cells, we tested the ability of exogenous IGFBP-2 to alter apoptosis induced by various chemotherapeutic agents. As both these cell lines produce large amounts of IGFBP-2, we also examined the effect of silencing IGFBP-2 using siRNA.
Results In MCF7 cells, paclitaxel (50 μM) increased cell death from 9% to 24.5% (P <0.001). Addition of IGFBP-2 (25 ng/ml) decreased the induced cell death by almost one-half to 17.7% (P = 0.047). 5-Fluorouricil (20 μM) increased cell death of T47D cells by 54% (P = 0.044), which was completely blocked by the addition of IGFBP-2. Conversely, loss of IGFBP-2 enhanced chemotherapy-induced apoptosis in both cell lines compared with a nonsilencing siRNA. We also observed that loss of IGFBP-2 reduced cellular proliferation – the live cell number decreased 73% (P <0.001) and 33% (P <0.001) in the MCF7 and T47D cells, respectively. Additionally, in the MCF7 cells, loss of IGFBP-2 alone increased cell death threefold (P <0.001).
Conclusions These data show that the production of IGFBP-2 by breast cancer cells enhances their survival and protects them against chemotherapy. Thus in breast tumours the increase in IGFBP-2 production could be a survival mechanism making IGFBP-2 a legitimate target for intervention.
Acknowledgements This research was funded by Breast Cancer Campaign.
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P23

Clinicopathologic and molecular predictors of axillary lymph node metastasis in early-stage breast cancer: a mathematical predictive model

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Introduction Axillary nodal (LN) stage is the most important prognostic factor in early-stage breast cancer (BC) which has risen as a result of widespread BC screening. However, surgical procedures for LN staging carry the risk of early and long-term postoperative morbidity. Therefore, reliable predictors of nodal status are needed to reduce the extent of axillary surgery and its consequences.
Materials and methods Predictive factors of axillary LN status at the time of primary diagnosis have been assessed using a broad panel of immunohistochemical tissue markers in a well-characterised series (n = 1,130) of primary operable (stage I & II) invasive BC cases, temporally divided into training (n = 730) and validation (n = 400) sets. Potential predictor factors were initially assessed using univariate analysis. A multivariate logistic regression model was fitted using backward stepwise variable selection in the training set. The resulting model was subsequently validated utilising the validation set.
Results Within the training set, the proportion of cancers with positive nodes was significantly higher with younger age, larger tumour size, higher grade, no special type tumours, definite vascular invasion (VI), ER^-, $HER2^+$, $PIK3CA^+$, and high Ki-67 Labelling Index (Ki-67LI). A multivariate logistic regression model indicated that predictors of nodal positivity included definite VI, higher grade, histological type, tumour size ≥2 cm, $HER2^+$, and Ki-67LI. This model resulted in 86.6% accuracy in predicting node positive cases, with area under the curve (AUC = 73.1%) and excellent goodness of fit (P = 0.981). Model cross-validation revealed an AUC of 72.3%.
Conclusions In this study, VI and tumour grade were the strongest independent predictive factors of nodal status in BC patients at the time of primary diagnosis. Our predictive model, which jointly incorporates VI, tumour grade, histological type, tumour size, HER2 status and Ki-67LI, confers an objective predictive accuracy relative to single predictive factors.
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P24

Assessing the functional role of caspase-8 gene variants in breast cancer

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Rationale and hypothesis Mutations in high-penetrance genes such as BRCA1 and BRCA2 predispose to breast cancer, and recently a number of low-penetrance breast cancer genes have also been identified. We reported that a coding SNP in the caspase-8 gene (CASP8 D302H) is associated with a reduced risk of breast cancer. More recently we identified a CASP8 4-SNP haplotype associated with an increased risk of breast cancer [1]. A CASP8 promoter indel has been associated with breast cancer in an Asian population, although this has not been confirmed in Europeans. Our hypothesis is that these CASP8 variants may influence breast cancer susceptibility via effects on the apoptotic response.
Objective Our objectives are to study the functional effects of six relevant CASP8 variants on caspase-8 activity and apoptosis induction in peripheral blood lymphocytes (PBLs).
Methods We recruited 68 healthy women attending the Sheffield Mammography Screening Service and measured the ability of their PBLs to undergo drug-induced apoptosis. Levels of apoptosis and caspase-8 activity were determined by fluorescence-activated cell sorting analysis (Annexin V-FITC with PI and Red-IETD-FMK (Calbiochem), respectively). The six SNPs were genotyped using TaqMan assays (Applied Biosystems). Data were analysed using parametric and nonparametric analysis of variance.
Results The median levels of induction of caspase-8 activity and apoptosis were 70.03% (28.19 to 94.65) and 78.11% (18.57 to 92.99), respectively. The rare alleles of rs3834129 (promoter indel), rs7608692 (intron 2) and rs1861269 (intron 4) were associated with reduced caspase-8 activity (P ANOVA = 0.03, 0.005 and 0.048, respectively). In addition, rs1861269 was significantly associated with reduced apoptosis (P ANOVA = 0.036). Although these results need to be confirmed, they suggest that SNPs in the caspase-8 gene may influence breast cancer susceptibility via effects on caspase-8 activity and apoptosis.
Reference
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P25

Polymorphisms, endogenous hormone levels and familial breast cancer risk in premenopausal women

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Introduction Epidemiological studies provide strong evidence of a role for endogenous sex hormone levels in the aetiology of breast cancer [1] and

suggest that levels may be partly genetically determined. Quantification of hormone levels in premenopausal women, however, is difficult because of their cyclical nature. In particular, oestrogen has a marked peak in the follicular phase and a further wider peak in the luteal phase.

Methods We developed a protocol to capture peak follicular phase urinary oestrone glucuronide (E1G), and luteal phase E1G in healthy premenopausal women. Repeated measurements of creatinine-adjusted E1G levels, in 789 women, were used to describe features of the E1G curve such as mean and peak follicular E1G, and luteal E1G. A total of 691 tagging SNPs capturing common variation in genes within the oestrogen synthesis and metabolism pathways were successfully genotyped. Geometric mean urinary E1G levels and endogenous plasma hormone levels were estimated and tested for an association with the genotype of each SNP.

Results We identified a rare SNP (minor allele frequency 7%), in which the minor allele was associated with a 20% reduction in circulating levels of E1G in healthy premenopausal women ($P < 10^{-8}$). We are currently genotyping this SNP in 12,000 breast cancer cases and 12,000 controls to test whether the reduction in circulating oestrogen levels is also associated with a reduction in breast cancer risk.

Conclusions Circulating hormone levels in premenopausal women may provide a useful intermediate phenotype in the search for low-penetrance breast cancer risk alleles.

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P26

A randomised controlled trial of the psychoneuroimmunological effects of reflexology in women with early-stage breast cancer

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Objective To evaluate the psychoneuroimmunological effects of reflexology in women with early breast cancer.

Methods One hundred and eighty-three women with early breast cancer were randomised 6 weeks post surgery to self-initiated support (SIS) (comparator intervention), SIS plus reflexology, or SIS plus scalp massage (control for physical and social contact). Patients randomised to reflexology and massage had eight sessions at weekly intervals. Primary and secondary endpoints were 18 and 24 weeks post surgery, respectively. Mood, coping and quality of life were assessed pre-randomisation, and at the two endpoints. Blood was also taken at these three time points to enumerate lymphocyte subsets (CD profiles), cytokine production (Th1, Th2), and hormones (prolactin, cortisol, growth hormone).

Results At week 18, massage, but not reflexology, was significantly better than SIS in terms of the primary outcome measure, the Trial Outcome Index (TOI) of FACT-B. At the secondary endpoint (week 24), reflexology, but not massage, was better than SIS in terms of the TOI. Lymphocyte phenotyping found that $CD25^+$ cells were significantly higher in the massage group compared with the SIS group at week 24. The percentage of T cells, more specifically the T-helper subset expressing IL-4, decreased significantly in the massage group compared with the SIS group at week 24. An accompanying increase in the percentage of $CD8^+$ cytotoxic T cells expressing IFNγ in the massage group showed that the immunological balance of patients can be altered in a potentially beneficial manner by massage. Neither reflexology nor massage affected any of the lymphocyte subsets, or hormones.

Conclusions Reflexology and massage have modestly beneficial effects on quality of life. Massage showed statistically significant effects on immunological parameters, although the clinical significance of these effects will require further investigation.

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P27

An integrated informatics platform to facilitate transforming tissue into knowledge

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Tissue banks provide a core facility that enable translational research to be undertaken on quality-assured patient-donated samples. These samples require clinical and pathological annotations to allow comparisons to be drawn between the research-generated data and the conventional pathological and clinical outcome parameters. The challenge is to develop a flexible database system to capture these parameters and to provide a statistical framework for automated analysis.

Various database solutions have been developed to capture the data at the numerous stages of research. A tissue banking platform allows complete tracking of samples as they are frozen, extracted and released, combined with an independent pathology database to capture the pathological and clinical outcome data for all breast cancer patients. This web-based interface allows the appointed research nurse to submit data as they are approved at the Multi Disciplinary Team Meetings. The patient records are also routinely checked to capture any data relating to disease recurrence, treatments and surgical procedures. A digital pathology database is also used to capture and store the tissue microarray results. All of these databases are then combined using another locally designed system, INSPIRE, which brings all the data together to perform statistical analyses and present the results via a user-friendly, web-based interface.

The various systems developed allow tissue-banked samples to be tracked throughout the research process and to be annotated with high-quality pathology and research-derived data. INSPIRE then completes the cycle by aggregating these data to perform statistical analyses and present these in a user-friendly, web-based interface. This allows the researcher to focus purely on generating high-quality research and enhancing our knowledge in breast cancer.

P28

Effect of intermittent versus continuous energy restriction on weight loss and breast cancer risk biomarkers

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Introduction The problems of adherence to energy restriction in humans are well known. Animal data suggest intermittent energy restriction (IER) is comparable with or better for preventing breast cancer than continuous restriction (CER).

Objective To compare the feasibility and effectiveness of IER with CER for weight loss and improving insulin sensitivity and other markers of breast cancer risk.

Methods Randomised comparison of a 25% energy restriction as IER (~2,266 kJ/day for 2 days/week) and CER (~6,276 kJ/day for 7 days/week) in 107 overweight or obese (mean ± SD body mass index = 30.6 ± 5.1 kg/m^2) premenopausal women over 6 months. Weight, anthropometrics, blood markers for risk of breast cancer and other metabolic diseases, insulin resistance, oxidative stress markers, leptin, adiponectin, lipids, inflammatory markers (high-sensitivity C-reactive protein and sialic acid), insulin-like growth factor-I and insulin-like growth factor binding proteins, androgens, prolactin and menstrual cycle length were assessed at baseline, 1, 3 and 6 months.

Results Eighteen subjects withdrew before 6 months (11 IER, seven CER). Last observation carried forward analysis showed IER and CER are equally effective for weight loss: mean (95% CI) weight change for IER was –6.4 (–7.9 to –4.8) kg vs. –5.6 (–6.9 to –4.4) kg for CER (P for difference between groups = 0.4). Both groups had significant and comparable improvements in disease risk markers; however, IER was significantly better than CER in reducing insulin resistance: mean (95% CI) change for IER was –28 (–37 to –17)% vs. –15 (–24 to –4)% for CER.

Conclusions IER is as effective as CER for weight loss and biomarker change. Its additional beneficial effect on insulin sensitivity indicates that it may be an alternative approach for weight loss.

P29

Thioredoxin and related redox systems as targets in breast cancer

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Redox systems are often deregulated in cancer. To investigate whether altered expression predicted response to therapy, core biopsies from 80 locally advanced breast tumours (pre-six cycles of fluorouracil epidoxorubicin cyclophosphamide/fluorouracil adriamycin cyclophosphamide chemotherapy) were stained, using standard immunohistochemistry, to examine members of the Trx system (Trx1, TrxR and TxNIP), GST-π, GST-θ, catalase and MnSOD, and results were correlated with clinicopathological criteria. Significant results were obtained between TxNIP and progression-free survival ($P = 0.008$) and overall survival ($P = 0.05$), with low expression predicting a worse prognosis. A redox protein profile was developed, using an artificial neural network approach, with four of the proteins (catalase, GST-θ, GST-π and TxNIP), that stratifies patients into good/poor prognostic groups for overall survival with an 88% sensitivity and 87% specificity.
Conventional *in vitro* studies show that, using MCF-7 cells, targeting the Trx system by pretreatment with novel inhibitors (PMX464, PMX290 or IV-2) sensitises resistant cells to conventional C/T but that sensitivity of the parental line remains unchanged. Initial results, using single agents in novel three-dimensional (3D) systems, shows differential chemosensitivity, between normal and malignant cells, that is not apparent using conventional two-dimensional (2D) systems. Parental cell lines (MCF-7, MDA-MB-231) maintain or become more sensitive when exposed in 3D to conventional chemotherapy and Trx inhibitors (doxorubicin, PMX464/PMX290, IV-2 that is IC_{50} 2D and 3D 0.01 μM, 0.5 μM, 25 μM – paclitaxel IC_{50} 3D <0.01 μM, 2D 0.01 μM), assessed by clonogenic survival. Normal breast epithelial cells (MCF10As), however, show increased resistance to drugs (paclitaxel, doxorubicin, PMX464/PMX290, IV-2 IC_{50} 3D 0.1 μM, 1 μM, >10 μM, 200 μM – IC_{50} 2D 0.05 μM, 0.01 μM, 0.5 μM, 25 μM, respectively). The reasons for such altered chemosensitivity in 3D matrices are a focus of current work.
Results from the immunohistochemistry and *in vitro* studies suggest the suitability of targeting redox proteins, particularly the Trx system, in breast cancer to improve the efficacy of conventional therapies.

P30

Could side population cells be an indicator of progression to hormone nonresponsive breast cancer

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There is increasing evidence that recurrent metastatic breast cancer arises in part due to the presence of long-lived, slow-cycling, and drug-resistant stem cells. Adult stem cells, known as side population (SP) cells, whose phenotype has been demonstrated to be due to the expression of ABCG2, are known to be resistant to a number of structurally unrelated compounds. In the present study we have observed that while both oestrogen-responsive and non-oestrogen-responsive breast cancer cell lines contain SP, exhibit multidrug resistance and express elevated levels of ABCG2, the non-oestrogen-responsive more highly metastatic MDA-MB-231 SP cells exhibit higher levels of mitoxantrone resistance than the other cell populations examined. These SP cells would therefore have a higher survival capacity when exposed to many currently utilised therapeutic regimes. Importantly, we have shown there is a statistically significant relationship between the presence of these SP cells in fine needle aspirates associated with ER-negative palpable breast lesions, and that these cells are more frequently associated with triple-negative breast tumours. Novel treatments directed against SP cells should be sought to offer patients better treatment strategies in these triple-negative tumours that fail to respond to conventional targeted therapy. Further analysis of this small population of potentially important cells is warranted.

P31

A novel tumour-based test to identify breast cancer due to BRCA1 and BRCA2 mutations

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Objective To develop a sensitive and specific pathology-based predictor to improve identification of BRCA1 and BRCA2 gene carriers.
Methods We assembled a training panel of breast cancer tumour blocks from 67 BRCA1, 71 BRCA2 associated and 105 sporadic young onset cases (≤40 years at diagnosis) from the Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) study. Gene carriers were matched to sporadic cases for ER status. Tissue microarrays were assembled and subjected to immunohistochemical analysis with a panel of 18 antibodies. DNA from tumour tissue and matched patient lymphocytes was subjected to high-resolution tiling path microarray-based comparative genomic hybridisation (aCGH). Bio-informatics analysis highlighted DNA regions significantly differentially lost, gained or amplified in BRCA1 or BRCA2 carrier tumours compared with controls. Chromogenic *in situ* hybridisation (CISH) identified amplifications in all training samples.
Results Two neighbouring regions of differential amplification (3q25.31 and 3q25.2) were identified in BRCA1 cases and one in BRCA2 cases (20q13.13). As expected, ER, PR and HER2 negative status was highly predictive of a BRCA1 gene carrier. Using just ER and HER2 plus the CISH probes we were able to assign BRCA1 and BRCA2 cases accurately in 74% and 81% of cases tested. The probability of misclassifying a control as a carrier was 5% and 12% in each case. These results equated to positive and negative predictive values of 0.92 and 0.90 for BRCA1 and 0.72 and 0.92 for BRCA2. The BRCA1 and BRCA2 tumour tests are being validated in a new set of tissue microarrays comprising 223 tumours from the POSH study.
Conclusions This tumour-based predictor for BRCA1 and BRCA2 carriers may prove useful to identify gene carriers at low *a priori* chance of having a mutation, to direct BRCA1/2 targeted treatment approaches and to identify familial non-BRCA1/2 cases that may be suitable for new gene discovery studies.

P32

Melanoma-associated antigen family protein-D4: clinical significance and functional relevance in breast cancer

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Melanoma-associated antigen (MAGE) family genes are broadly expressed during development and are involved in the regulation of cell survival, cell cycle progression and apoptosis. MAGE family proteins are generally described as tumour-specific antigens and as representing ideal targets for cancer immunotherapy. In the current study, we identified melanoma-associated antigen protein-D4 (MAGE-D4), a recently characterised MAGE family member, as a new prognostic biomarker and potential therapeutic target for breast cancer. Specifically, in a whole genome microarray analysis of 103 cases of invasive breast tumours, MAGE-D4 expression was observed in 43.8% of tumours, while undetectable in normal breast tissue. Multivariate and univariate analyses also indicated MAGE-D4 expression to be associated with tumour grade, spread to lymph nodes and shortened times to relapse ($P = 0.0369$) and death ($P = 0.0133$) from time of cancer diagnosis; suggesting a role for MAGE-D4 in tumour progression. To further investigate the involvement of MAGE-D4 in breast cancer cell biology, the phenotypic effects of this gene were characterised *in vitro*. We observed a marked upregulation of MAGE-D4 expression – at both mRNA and protein levels – in the breast cancer cell line Hs578T compared with the

syngenic Hs578Bst breast cell line. Interestingly, RNA interference-mediated knockdown of MAGE-D4 expression in Hs578T cells significantly reduced cell migration and invasion and correlated with inhibition of STAT3 and NF-κB p65 subunit phosphorylation, thus affecting two common signalling pathways involved in regulating cancer progression. Moreover, monolayer cell growth rate was not affected by MAGE-D4 gene knockdown, while growth in soft agar was significant compromised. Our results indicate that MAGE-D4 contributes to the tumorigenesis of breast cancer cells by regulating migration, invasion and anchorage-independent growth, and therefore may represent a novel target for the detection and treatment of breast cancer.

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P33

Identification of molecular subtypes within a formalin-fixed, paraffin-embedded breast cancer tumour cohort

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Breast cancer is not a single disease but is highly heterogeneous at both the molecular and clinical levels. Gene expression profiling of breast tumours by multiple independent groups and technologies have revealed five major molecular subtypes of breast cancer. These molecular differences result in distinct clinical outcomes and responses to treatment.

The gene expression profiling studies that have defined the molecular subgroups of breast cancer to date were performed using fresh frozen tissue. Routine clinical practice dictates the preservation of surgical specimens via paraffin embedding of formalin-fixed tissue. Therefore, derivation of molecular subgroups of breast cancer from formalin-fixed, paraffin-embedded (FFPE) preserved tissue would have more application in the clinical setting as such profiles could be applied to routinely collected specimens.

The Almac Diagnostics' Breast Cancer DSA™ has been optimised for analysis of FFPE samples enabling the use of these valuable archived tissue banks. We have demonstrated the ability to identify the molecular subgroups previously defined within a cohort of sporadic, BRCA1 mutant and BRCA2 mutant FFPE breast tumours as well as defining two novel subgroups within this tumour set. This study demonstrates that it is possible to derive biologically meaningful data from a cohort of archived FFPE tumour samples using the Almac Breast DSA™. We demonstrate that there is considerable molecular diversity within BRCA mutant and sporadic breast tumours, suggesting that traditional assumptions of the behaviour of tumours based on their immunohistochemistry status may not always be correct. At present, a number of clinical trials are stratifying patients for poly(ADP-ribose) polymerase 1 (PARP-1) inhibitor therapy based on BRCA mutation and triple-negative status. The data presented here would suggest that not all BRCA1 mutant, BRCA2 mutant and indeed triple-negative patients are similar at the molecular level and as such will not respond equally to PARP-1 inhibitor or indeed other therapeutics in the same manner.

P34

Evaluating gene expression in formalin-fixed, paraffin-embedded breast cancer tissues using DASL®

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The study of gene expression in conventionally processed tissues is hampered by degradation of mRNA. Expensive, low-multiplex, quantitative PCR methods can be unreliable due to the limited template sizes. One way to overcome this problem is to use array-based methods. DASL® technology relies on random priming for production of cDNA, in concert with universal bead arrays to allow the detection and relative quantitation of expression of specific gene subsets.

Using the Illumina DASL® Cancer Panel (500 cancer-associated genes on one array), we evaluated the expression of key genes in archival formalin-fixed, paraffin-embedded tissue samples from 80 breast cancer patients with well-characterised pathological and clinical features. We first assessed transcript integrity in the samples on the basis of levels of mRNA encoding *RPL13A*, prior to running the Cancer Panel. A subset of genes of interest was then assessed by quantitative PCR to confirm the relative levels observed using the DASL® assay. Finally the expression of the same subset of genes was evaluated at the protein level by immunohistochemistry.

We were able to predict, with good accuracy based upon *RPL13A* assays, those samples unsuitable for DASL® analysis. Furthermore, the results of DASL® analysis showed good correlation with protein levels, as measured by immunohistochemistry, for a number of key genes including *ERBB2* (HER2) and *ESR1* (ER). We conclude that DASL® represents a powerful tool for assessing expression of multiple genes in archival tissue.

P35

miR-433 overexpression attenuates the spindle assembly checkpoint response to paclitaxel

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Paclitaxel is a microtubule inhibitory chemotherapeutic drug that is increasingly used for the treatment of solid tumours. *In vitro* studies have demonstrated that attenuating the spindle assemble checkpoint (SAC) alters the post-mitotic responses to paclitaxel. Furthermore, the aberrant expression of a number of the SAC proteins, MAD2, BUBR1, and Aurora A kinase, are associated with poor patient prognosis. We have identified a microRNA, miR-433, that regulates the expression of MAD2. Overexpression of miR-433 in Hela cells induced downregulation of MAD2 mRNA and protein expression. We have also shown that Hela cells overexpressing miR-433 and treated with paclitaxel are no longer capable of cyclin B stabilisation, and thus have lost the ability to activate the SAC in response to paclitaxel. In addition, cell viability assays showed that Hela cells overexpressing miR-433 and treated with paclitaxel have an attenuated response to paclitaxel compared with microRNA scrambled controls. We have characterised the levels of miR-433, MAD2 gene expression and MAD2 protein levels in a cohort of ovarian cancer cell lines. Cell viability assays on this cohort revealed that responsiveness to paclitaxel is associated with high MAD2 protein expression and lower miR-433 expression. We hypothesise that the expression of miR-433 when deregulated in cancer leads to altered MAD2 expression and a compromised SAC, a key feature underlying drug resistance to paclitaxel. In a pilot study of paired human breast tumour and normal breast tissue samples we have shown that expression levels of miR-433 are elevated in cancer tissue. Targeting this microRNA in cancer may improve the efficacy of paclitaxel in treating breast cancer and ovarian cancer.

P36

Breast calcification: the 'Cinderella' breast element?

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Introduction There is currently extensive diagnostic use of breast tissue calcifications through their differential mammographic appearance, albeit with relatively low specificity. However, the details of the calcification chemical and structural composition remain somewhat vague. Thus any associated clinical significance, such as indications of tumour type, grade and stage, have not previously been explored.

Methods The biochemical composition and incorporation of carbonate into the hydroxyapatite lattice of type II microcalcifications was studied by infrared microspectroscopy, allowing spectral information to be directly correlated with associated histopathology of the surrounding tissue.

Results It was shown that the chemical characteristics of calcifications associated with benign, *in situ* and invasive pathologies are significantly different. For the first time, a relationship between grade of pathological breast disease and chemical nature of associated microcalcifications has been demonstrated. In particular we have found significant correlations between distinct pathology grades and physiochemical features such as the carbonate content of microcalcifications and protein to mineral ratios. Further, such correlations were also demonstrated within carcinoma *in situ* and invasive cancer subgrades. Quantification of the calcification carbonate content indicated that the degree of carbonate substitution followed a monotonic trend between benign, ductal carcinoma *in situ* (DCIS) and invasive pathologies (see Figure 1). This suggests that benign tissue calcification (consisting

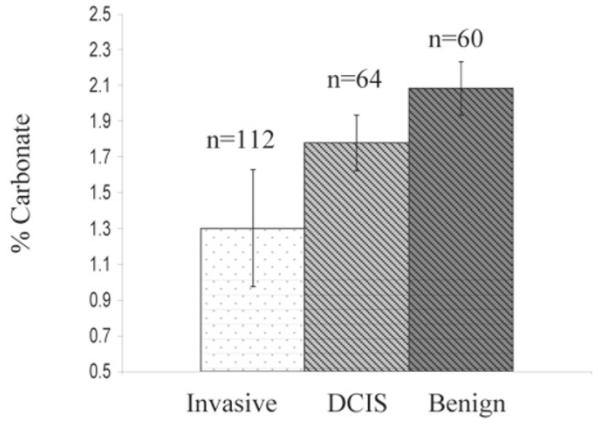

Figure 1 (abstract P36). Apatite $\%CO_3^{2-}$ for each pathology group.

of fibroadenoma, ductal hyperplasia and fibrocystic change) is likely to lead to a DCIS, which in turn will result in invasive disease.

Conclusions This study a greater significance for microcalcification chemistry in mechanisms associated with cancer progression, and especially for the future diagnosis and classification of breast pathology.

P37

Expression of migration stimulating factor in breast tissues and its clinical significance

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Introduction Migration stimulating factor (MSF) is a novel angiogenic factor previously identified in breast tumours and their associated stroma. The aim of this study was to determine the possible diagnostic and prognostic value of MSF expression in these tumours and its effects on breast-derived cells *in vitro*.

Methods Paraffin-embedded archival breast tissues were stained with specific MSF antibodies and the level of staining was semiquantified either by consensus of two or three independent observers or by computer-assisted image analysis. The effects of rhMSF on the migration and proliferation of breast carcinoma cells, fibroblasts and endothelial cells were examined in tissue culture.

Results MSF expression was generally low or negligible in normal breast tissue derived from reduction mammoplasties (NB; n = 19). However, histologically normal breast from the resection margin of breast tumours (NB-T; n = 17) showed significantly higher expression than NB. Significant increases in MSF expression were also observed from NB to benign lesions (B; n = 8) and from any of these tissues (B, NB or NB-T) to malignant tumours (T; n = 23), whereas B and NB-T showed similar expression.

MSF was detected in approximately 85% of the tumours examined, being heterogeneously expressed in carcinoma cells as well as in fibroblasts and blood vessels. In a cohort of 71 tumours, high MSF expression was associated with larger tumour size and shorter patient overall survival. Stromal MSF produced the most significant results. Recombinant MSF stimulated the migration, but not the proliferation, of breast carcinoma cells, fibroblast and endothelial cells.

Conclusions This study indicates that MSF expression is associated with breast tumour development and aggressiveness. Besides inducing angiogenesis, MSF acts as an autocrine and paracrine motogen in breast tissues.

P38

Lack of correlation between markers of breast cancer initiating cells

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Breast Cancer Research 2010, **12(Suppl 1):**P38 (doi: 10.1186/bcr2535)

Introduction The existence of breast cancer-initiating cells was initially demonstrated by Al-Hajj and colleagues [1] using antigen expression, and subsequent studies have employed several methodologies to identify and isolate these cells. However, there are limited data describing whether similar cell populations are recognized by the different approaches.

Materials and methods Using breast cancer cell lines MCF7, MDAMB231 and MDAMB468, we have compared the antigen expression profile ($CD44^+CD24^{-/low}$) against the side population and the ability to form tumour spheroids. Immunostaining on cells and xenografts was also performed to search for expression of potential stem cell markers.

Results Our data showed increased $CD44^+CD24^{-/low}$ population in both MCF7 and MDAMB468 spheroids, but growth advantage was only observed in sorted MDAMB468 $CD44^+CD24^{-/low}$ cells. In contrast, analysis of the antigen profile of the side population did not demonstrate any correlation and no growth advantage was found in sorted MCF7 and MDAMB468 cells. Immunostaining of MCF7-derived tumour xenografts showed two potential markers, p63 and sox2, in addition to CD44; both MDAMB231 and 468-derived xenograft expressed strong CD44, and the latter was also stained for p63 and aldehyde dehydrogenase (ALDH). In addition, comparison between the antibodies only demonstrated partial overlap between CD44 and p63/ALDH in MCF7 and MDAMB468 xenografts. Therefore, in MCF7/MDAMB468-like breast tumours, p63 and sox2/ALDH recognize different stem/progenitor cell populations, and the combination of CD44 and p63/ALDH further clarifies the boundary of these cells.

Conclusions Our results indicate that each breast cancer is unique, and therefore tumour-initiating cell markers and methodologies should be applied specifically.

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P39

Mitochondrial translocator protein modulates metabolism and pharmacologically induced apoptosis in breast cancer cells

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Introduction Dysfunctional mitochondria contribute to the onset of malignant transformation and growth. Molecules that regulate mitochondrial homeostasis are therefore the object of great attention to identify novel therapeutic strategies. The mitochondrial translocator protein (mTSPO) stands in a critical position for mitochondrial homeostasis and is involved in the physiology of breast cancer where it is overexpressed and positively associated with aggressiveness [1]. mTSPO ligands are therefore exploited for cancer imaging and chemotherapy, such as PK11195. mTSPO is associated with the voltage-dependent anion channels (VDACs), which regulate the metabolites' flux into mitochondria [2]. mTSPO expression is driven by the oncogene protein kinase Cε, suggesting a fundamental crosstalk for malignant transformation and uncontrolled proliferation. We hypothesized that mTSPO by regulating VDAC performance impinges on metabolism and pharmacologically induced cell death in breast cancer cells.

Results In human breast adenocarcinoma MCF-7 and in cervical cancer cells (HeLa) we found, via imaging and luminescent-based approaches, that a decreased mTSPO/VDAC ratio of expression uperegulates mitochondrial Ca^{2+} uptake and ATP generation whilst reducing the rate of reactive oxygen species generation calling for a metabolic switch via an improvement of mitochondrial function. mTSPO suppression also impairs protein kinase Cε activation and facilitates Ca^{2+}-dependent apoptosis triggered by C_2-ceramide. Nevertheless, mTSPO targeting with PK11195 – which impinges on Ca^{2+} homeostasis [3] – raises C_2-ceramide cell death in MCF-7 and in the more aggressive line of adenocarcinoma MDA10 – characterized by an increased mTSPO/VDAC ratio of expression.

Conclusions The evidence proposes mTSPO as a neglected pathway in the cell signalling of breast cancer and paves the way for future studies to exploit mTSPO as a suitable prognostic marker and target for molecular chemotherapy.

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P40

Identification of proteins and signalling pathways involved in neoadjuvant chemotherapy responsiveness of breast tumours using proteomics

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Introduction A significant proportion of patients presenting with large locally advanced breast tumours, treated with neoadjuvant chemotherapy (NAC), show a poor or partial response to the treatment. The aim of this study is to identify novel phospho-proteins that may predict responsiveness to NAC treatment as there are no such biological markers available in the clinic to date.

Materials and methods Frozen tissues collected before (core biopsies) and after NAC (surgical tissues) were categorised by pathological response (complete response, no response and progressive disease). Lysates were enriched for tyrosine phospho (pY) proteins and separated in two dimensions by IEF and PAGE. Proteins showing consistent differences in tyrosine phosphorylation from the different response groups were identified by mass spectrometry (MALDI-TOF) and using the NCBInr sequence database (ProFound). Functional and pathway analysis was performed using Ingenuity Pathway Analysis (www.ingenuity.com).

Results Phospho-protein expression profiles were successfully established from core biopsies and surgical tissues. Proteins involved in cell division, polarisation and microtubule formation such as PAR6D and Kif3 were identified in core biopsies from the complete response group. In the no response/progressive disease group, proteins such as CHIMP, ZAP70, and serologically defined breast cancer antigen (NY-BR-15) were distinguished. Further network pathway analysis in this disease group suggested that two main signalling pathways, TP53 and TNF, may be involved in NAC nonresponsiveness.

Conclusions Proteins and pathways were identified that showed scientific and clinical relevance to NAC responsiveness. Our data support the largely conflicting evidence that suggests P53 and TNF may be possible predictive markers for NAC responsiveness. These findings may lead to the accurate prediction of chemotherapy responsiveness in breast cancer patients and to the development of personalised treatment plans for future patients.

P41

Reduced MCPH1 expression in breast cancer and response to chemotherapy

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Introduction Previously we identified MCPH1, a DNA damage response protein involved in the regulation of *BRCA1* and *BRCA2*, as the defective protein in one form of microcephaly. *BRCA1* mutations are associated with basal-like breast cancer, which are often also negative for oestrogen receptor (ER), progesterone receptor (PR) and HER2. Our data indicate that MCPH1 plays a role in response to chemotherapeutic agents used in the treatment of breast cancer due to its role in DNA repair and the spindle checkpoint.

Methods MCPH1 immunohistochemistry was performed on 320 breast cancers and correlated with pathology, survival, ER, PR and HER2 data. Drug assays were performed on the breast cell lines MCF10A, MCF7 and HCC193 with different MCPH1 and BRCA1 backgrounds created using siRNA.

Results We identified reduced MCPH1 expression in 23% (74/320) of breast cancers. After performing continuous data analysis, the mean MCPH1 expression decreased with increasing grade, grade 1 and 2 versus grade 3 (P <0.006). Interestingly, mean MCPH1 expression was also lower in ER/PR-negative (P <0.001) and triple-negative cancers (P <0.004). An association with HER2-positive cancers was also identified (P <0.03). While no association with survival was identified initially, the longer term survival was better in patients with higher mean MCPH1 expression. Our cell line drug assays indicate that MCPH1 plays a role in resistance to Taxol and sensitivity to cisplatin and doxorubicin.

Conclusions MCPH1 expression is reduced in 23% of breast cancers, particularly in higher grade tumours. Interestingly, reduced mean MCPH1 expression was associated with the triple-negative phenotype often seen in basal-like cancers. Further basal cell markers are currently under investigation. Aggressive basal-like breast cancers have a poor prognosis; MCPH1 expression may potentially improve treatment of these cancers.

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P42

Anti-HER2 imaging agents for breast cancer imaging

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Introduction Overexpression of human epidermal growth factor receptor 2 (HER2) tyrosine kinase cell surface receptor occurs in 25 to 30% of all breast cancer and is linked to aggressive phenotype and high-mortality disease. HER2 is a clinically important target in diagnosis and treatment of breast cancer but despite its pivotal role there are no established tools for quantitative clinical imaging of the extent and location of HER2-positive ($HER2^+$) tumours in patients. Such a tool could provide important clinical diagnostic information by early detection of subclinical $HER2^+$ disease, optimal management of current anti-HER2 therapies and response assessment of novel therapeutics. We aimed to generate recombinant proteins that would achieve sensitive and specific detection of $HER2^+$ tumours in the clinic using radioimmunoimaging.

Materials and methods Two different HER2-binding molecules were investigated: C6.5 a small dimeric antibody fragment (diabody), which is approximately 1/3 of the size of an antibody; and G3, a small monomeric designed ankyrin repeat protein (DARPin) that is 1/10 the size of an antibody. The agents were generated in the yeast *Pichia pastoris* system using processes compliant with good manufacturing practice (GMP). C6.5 and G3 production strains were constructed to allow methanol-inducible, soluble expression. The expressed proteins were purified using expanded-bed adsorption–immobilized metal affinity chromatography.

Results and conclusions For C6.5 the final product was homogeneous, stable and free of host cell and other relevant contaminants. The protein was stable during storage, with no evidence of aggregation. In addition, affinity for HER2, as measured by Biacore analysis, was not compromised by storage at either 4 or –80°C. Preliminary results with the G3 DARPin indicate that this protein is also amenable to GMP production in *P. pastoris*. The relative efficacy of these agents for specific radioimmunoimaging of $HER2^+$ tumours *in vivo* is currently under investigation.

P43

Predicting interaction networks of breast cancer risk genes using multiple microarray data

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Introduction Global expression profiling by microarray can provide invaluable information about biological properties of breast cancers. Here I report predicted gene regulatory networks of known breast cancer risk genes using multiple microarray data, in order to understand how the risk genes interact with each other and how the interaction may be related to the pathogenesis of breast cancer.

Methods I used microarray data of breast cancer samples from four published studies. By combining the data from three smaller studies, I obtained two datasets with 548 and 684 samples, respectively. For each dataset, Pearson coefficients of expression levels between 74 known breast cancer risk genes were calculated first. The gene association network was also obtained by a new correlation metric called asymmetric correlation, which quantifies the

nonlinearity of the correlations. Finally the results from two analyses were combined to obtain predicted gene regulatory networks.
Results I found in both datasets that ESR1, GATA3 and FOXA1 formed a close cluster and each of them had interactions with a number of genes. In particular, FOXA1 showed positive interactions with ERBB2 and IGF1R while ESR1 and GATA3 were positively associated with NAT2 in both datasets. Positive associations were also found between AGTR1, FOXA1 and GATA3, and between CDH1, NAT2 and FGFR2. Moreover, FGFR2 and AGTR1 had negative associations with ERBB2, indicating that they have overwrapping but distinct gene network.
Conclusions Transcription factors ESR1, GATA3 and FOXA1 were found to form a core network, which was connected by plasma membrane signal transducers ERBB2, IGF1R and AGTR1. FGFR2 and CDH1 are associated with this network, but they seem to play distinct roles in breast cancers.

P44

Microarray based expression profiling of BRCA1 mutated human tumours using a breast-specific platform to identify a profile of BRCA1 deficiency

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Introduction The BRCA1 tumour suppressor gene is mutated in a significant proportion of hereditary breast cancer cases. Downregulation of BRCA1 mRNA and protein expression has also been reported in approximately 30% of sporadic breast cancer cases. BRCA1 is strongly implicated in the maintenance of genomic stability by its involvement in multiple cellular pathways including DNA damage signalling, DNA repair, cell cycle regulation, protein ubiquitination, chromatin remodelling, transcriptional regulation and apoptosis. Both pathological and gene expression profiling studies provide evidence that breast cancers with germline mutations in BRCA1 are different from non-BRCA1-related breast cancers.
Methods Extensive gene expression profiling and data analysis has been performed on a cohort of 70 formalin-fixed, paraffin-embedded-derived BRCA1 mutated breast tumours and matched sporadic controls using the Almac Breast Cancer DSA™ research tool. Validation of gene targets has been performed by quantitative RT-PCR and western blotting.
Results A list of differentially expressed transcripts has been derived from the comparison of these BRCA1 mutant breast tumours to matched sporadic controls. Functional analysis of this gene list was performed to identify the main pathways and processes that are deregulated by these transcripts. BRCA1 deficiency was associated with deregulation of pathways involved in: immune response, metastasis and invasion, cytoskeletal remodelling, spindle assembly and chromosome separation, and apoptosis and survival. We have now validated several panels of genes that characterise this BRCA1-deficient breast cancer profile. A high-throughput siRNA-based screening strategy will now be performed to identify functionally relevant BRCA1-associated gene targets involved in cell growth, differentiation and chemotherapy response.
Conclusions This approach has identified a set of transcripts that could be used to identify both hereditary and sporadic breast cancer patients with BRCA1 deficiency.

P45

FKBPL: a novel prognostic and predictive biomarker?

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Approximately 40% of patients with oestrogen receptor (ER)-positive breast cancers do not respond to endocrine therapies; furthermore, most responsive tumours eventually become resistant. We have identified a novel oestrogen-responsive Hsp90 co-chaperone and immunophilin, FKBPL, which affects the stability and signalling of ER with implications for breast cancer growth and sensitivity to endocrine therapies. MCF7 cells stably overexpressing FKBPL demonstrate a slower rate of proliferation and become highly dependent on oestrogen for their growth. This dependence on oestrogen renders these cells dramatically more sensitive to tamoxifen and fulvestrant. FKBPL overexpressing cells also exhibit decreased levels of ER and an oestrogen-responsive gene, cathepsin D, critical for breast cancer growth, survival and invasion. Moreover, knockdown of FKBPL using a targeted siRNA approach dramatically increases both ER and cathepsin D protein levels and cell resistance to tamoxifen. FKBPL has been previously implicated in the stabilisation of the cyclin-dependent kinase inhibitor, p21 [1]. Loss of p21 has been associated with a tamoxifen growth-inducing phenotype and hyperphosphorylation of ER at Ser118, with increased expression of ER-regulated genes. Following FKBPL knockdown, we have observed a fall in p21 levels and subsequent increase in Ser118 phosphorylation following treatment with 17β-estradiol or tamoxifen while FKBPL overexpressing cells exhibit the reverse effects. Our *in vitro* data support a model in which high levels of FKBPL would stabilise p21, decrease ER phosphorylation and abrogate tamoxifen-induced agonist potency, thereby increasing drug sensitivity, and suggest that FKBPL may have prognostic value that might impact upon tumour proliferative capacity and improve patient outcome. In addition, analysis of two publically available breast cancer microarray patient cohorts demonstrated that high FKBPL expression was correlated with increased overall and distant metastasis-free survival.

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P46

Stick or switch? Audit of the use of switch therapy from tamoxifen to an aromatase inhibitor in breast cancer

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Introduction Tamoxifen has an established role as the standard adjuvant therapy for early oestrogen-receptor-positive breast cancer. Aromatase inhibitors are now licensed for adjuvant treatment following 2 to 3 years of tamoxifen. This switch therapy offers both disease-free and modest overall survival advantages compared with 5 years of tamoxifen [1]. Greater Midlands Cancer Network guidelines based on NICE guidelines (2006) recommend switch therapy in patients who are not at low risk of recurrence [2]. There are no reports in the literature to indicate whether this is currently happening in clinical practice. We examined our own patient population to see if high-risk patients were being switched appropriately.
Methods Retrospective audit of all females diagnosed with invasive breast carcinoma between July 2006 and December 2007 at the University Hospital of North Staffordshire.
Results Of the 291 women diagnosed with invasive breast cancer, 13 fulfilled the inclusion criteria. Forty-six per cent of these were switched appropriately. In the remaining 54% of cases a switch had not been considered.
Conclusions More than one-half of the women receiving adjuvant tamoxifen are not being considered for a switch, which puts them at an increased risk of disease recurrence. Factors identified by the audit that could be modified to improve practice are: highlighting the tamoxifen start date in the patient notes to enable the reviewing clinician to more easily identify when a switch is due, and clearer ownership of ongoing adjuvant therapy between surgeons and oncologists.

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P47

Topoisomerase 2 alpha as a predictor of response to anthracycline neoadjuvant chemotherapy in locally advanced breast cancer

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Introduction Anthracyclines play an important role in the treatment of breast cancer but their beneficial therapeutic effects may not be the same for all

breast cancer patients. Side effects, including cardiotoxicity, may be avoided if biomarkers of response could be identified. Topoisomerase 2 alpha (Topo2α) is a target of anthracyclines and has been proposed as a chemosensitivity marker of anthracycline-containing therapies by *in vitro* and *in vivo* studies. But the method to detect it remains a controversial issue. Treatment in the neoadjvant setting is a unique opportunity to examine biomarkers of response to chemotherapy.

Objective To examine the role of Topo2α in response to anthracycline neoadjuvant chemotherapy both at the protein level by immunohistochemistry (IHC) and at the gene level using chromogen *in situ* hybridisation (CISH).

Patients and methods This study includes 100 locally advanced breast cancer patients treated by fluorouracil, epirubicin, and cyclophosphamide (FEC) chemotherapy from 1999 to 2008. Pretreatment core biopsy is used to study different biologic markers (basal cytokeratin, EGFR, Ki-67, p53, and Her2) including Topo2α by IHC and to correlate the expression level with the rate of pathological complete response (pCR). CISH is used to determine the level of Topo2α gene amplification.

Results In the initial analysis of the first 64 patients, the rate of clinical complete response (CR) and pCR were 40.6% and 19.7%, respectively. Prechemotherapy Topo2α protein expression correlated with both clinical CR ($P = 0.006$) and pCR ($P = 0.001$). On multivariate analysis, only Topo2α expression correlated with pCR ($P = 0.03$). A correlation between Topo2α protein expression and gene amplification will be made.

Conclusions The study demonstrates the importance of Topo2α as a predictor for both clinical CR and pCR to neoadjuvant anthracyclines in locally advanced breast cancer. Furthermore, it could be considered as part of pretreatment evaluation to help in improving the selection of patients for this type of treatment.

P48

CYP2D6 genotype affects outcome in postmenopausal breast cancer patients treated with tamoxifen monotherapy

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Introduction Tamoxifen efficacy may be influenced by a number of factors, including *CYP2D6* genotype, co-administration of drugs that inhibit CYP2D6, and adherence to tamoxifen therapy. CYP2D6 plays a major role in catalyzing the conversion of tamoxifen to its active metabolite endoxifen. Studies of the relevance of *CYP2D6* genotyping have had conflicting results due to various limitations: differences in *CYP2D6* allele coverage, phenotype classification and other confounding variables.

Methods Using archival samples from two UK cohorts of tamoxifen-treated women with invasive breast cancer (Dundee, $n = 391$; Manchester, $n = 227$), we estimated the association of inferred CYP2D6 metabolic phenotypes with recurrence-free survival time (RFS) using Cox proportional hazard models, adjusted for nodal status and tumor size. Comprehensive *CYP2D6* genotyping was performed using the AmpliChip CYP450 test.

Results Sixty percent of patients had at least one reduced-function *CYP2D6* allele and 6% had no functional alleles. Analysis of the entire group revealed a nonsignificant trend for worse RFS in patients with any reduced function alleles – HR = 1.52 (CI = 0.98 to 2.36, $P = 0.06$). In the subset of postmenopausal women on tamoxifen monotherapy, the HR for recurrence for patients with reduced functional alleles was 1.96 (CI = 1.05 to 3.66, $P = 0.036$). When the analysis was limited to four common allelic variants of *CYP2D6* [1], this difference was not apparent.

Conclusions This study indicates that patients with two fully functional *CYP2D6* alleles are more likely to experience the full therapeutic benefit of tamoxifen. The apparent adverse effect of reduced function alleles is best detected by a genotyping test with comprehensive *CYP2D6* allele coverage that captures uncommon variants with decreased function.

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P49

Recruitment of insulin receptor substrate-1 by erbB3 impacts on IGF-IR signalling in oestrogen receptor-positive breast cancer cells

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Insulin-like growth factor receptor (IGF-IR) signalling classically involves phosphorylation of insulin receptor substrate-1 (IRS-1) to recruit key downstream signalling pathways effecting breast cancer cell proliferation and survival. Recently, we have shown a further capacity for IRS-1 to associate with the epidermal growth factor receptor (EGFR/erbB1), with activation of EGFR promoting recruitment and phosphorylation of IRS-1 in an oestrogen receptor (ER)-positive tamoxifen-resistant breast cancer cell line. In this study, we examined recruitment of IRS-1 by another member of the erbB receptor family, erbB3, in three ER-positive breast cancer cell lines. Our studies revealed an interaction between erbB3 and IRS-1 in MCF-7, T47D and BT474 cells with HRGβ1 treatment significantly enhancing this recruitment and promoting IRS-1 phosphorylation at tyrosine (Y) 612, a specific phosphoinositide 3-kinase (PI3K) binding site. IRS-1 appears to play a key role in erbB3 signalling in MCF-7 and T47D cells as its knockdown using siRNA greatly impaired HRGβ1 signalling via PI3K/AKT in these cell lines. This novel interaction may have clinical relevance as immunohistochemical analysis of ER-positive breast cancer patient samples revealed IRS-1 Y612 expression positively correlated with total erbB3, p-AKT and Ki67 expression. Importantly, we found that recruitment 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, blockade of erbB3 signalling 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 breast cancer cells and this may provide an adaptive resistance mechanism when these receptors are targeted individually. Consequently co-targeting of IGF-IR and erbB receptors may prove to be a more effective strategy for the treatment of ER-positive breast cancer.

P50

Targeting DNA replication before it starts: Cdc7 as a therapeutic target in p53 mutant Her2 and triple negative breast cancer

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Based on protein expression profiles of core regulatory proteins involved in the G_1–S and G_2–M phase transitions, we have identified three distinct cell cycle phenotypes in a series of 200 breast cancers: a G_0 out-of-cycle state (18% of cases); a G_1 arrested/delayed state (24% cases); and accelerated S-G_2-M phase progression (58% of cases). The accelerated cell cycle progression phenotype had a higher risk of relapse when compared with G_0 and G_1-delayed/arrested phenotypes (HR = 3.90 (95% CI = 1.81 to 8.4), $P < 0.001$) and was associated with Her2 and triple negative subtypes ($P < 0.001$). High-grade tumours with the G_1-delayed/arrested phenotype showed an identical low risk of relapse compared with well-differentiated G_0 tumours. In addition to its prognostic significance, the cell cycle phenotype also impacts on individualised therapeutic decisions. Only patients showing the actively cycling, aggressive cell cycle phenotype are likely to benefit from conventional chemotherapeutic S-phase-directed or M-phase-directed agents or from the new generation of targeted cell cycle inhibitors that are now entering clinical trials.

The DNA replication initiation factor Cdc7 is an emerging anticancer target. Cdc7 inhibition results in an abortive S phase and potent cancer cell killing. Specificity is based on normal cells undergoing a reversible G_1 arrest following Cdc7 inhibition due to activation of a novel cell cycle checkpoint that is lost or impaired in cancer cells. Our analysis of the molecular circuitry underlying this replication origin activation checkpoint reveals that G1 arrest is dependent on three nonredundant checkpoint axes coordinated through the Forkhead transcription factor FoxO3a and p53. We show that only breast cancers displaying the accelerated cell cycle phenotype express elevated Cdc7 levels and are therefore highly represented in p53 mutant Her2-subtype and triple negative tumours. Breast cancers of the luminal subtype expressing low levels of Cdc7 undergo a cytostatic G_1 arrest after Cdc7 inhibition due to their p53

wild-type status, a checkpoint response mimicking untransformed cells. In contrast, Her2 and triple negative tumours show a marked response to Cdc7 inhibitors with potent cancer-cell-specific killing as a result of overexpression of the target protein and a result of impairment of the origin activation checkpoint due to p53 lesions.
We have thus defined a new therapy and a means of assessing response.

P51

Genetic engineering of pharmacologically regulated T cells, specific for breast cancer target antigens

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Peripheral blood T cells can be genetically targeted against cancer using fusion receptors known as chimeric antigen receptors (CARs). Many preclinical studies have provided great encouragement for this approach. However, pioneering clinical trials have been less successful and identified poor T-cell survival in patients as a crucial limiting factor. To address this, what is needed is a system to achieve selective expansion of tumour-specific effector T cells, both *in vitro* and *in vivo*. Here, we describe such an approach using IL-4, a pharmaceutical that has been tested in cancer patients and which is normally a poor mitogen for T cells. A chimeric cytokine receptor named 4αβ was engineered in which the IL-4 receptor α (IL-4Rα) ectodomain was fused to the shared β_c subunit, used by IL-2/IL-15. Addition of IL-4 to 4αβ-expressing T cells resulted in selective phosphorylation of STAT3/STAT5/ ERK, mimicking the actions of IL-2 or IL-15. Using receptor-selective IL-4 muteins, partnering of 4αβ with γ_c was implicated in these findings. Next, human T cells were engineered to co-express 4αβ with CARs specific for two breast cancer targets: MUC1 or the extended ErbB family. These T cells exhibited an unprecedented capacity to undergo IL-4-dependent expansion *in vitro* and repeatedly destroyed breast cancer cultures, greatly exceeding the performance of IL-2-stimulated cells. Importantly, 4αβ-expressing T cells retained cytolytic specificity for target antigen and dependence upon IL-4 (or IL-2) for survival. We have also used this system to achieve rapid IL-4-driven *ex vivo* expansion and enrichment of CAR^+ human T cells in bags (T-bags). Experiments were performed under closed and pseudo-good manufacturing practice conditions, scaling up for phase 1 clinical trials. T cells expanded in this manner demonstrate Th1 polarisation and potent tumour destructive activity, both *in vitro* and *in vivo*, in tumour-bearing SCID Beige mice. Together, these findings provide proof of principle for the development of pharmacologically regulated T-cell immunotherapy for breast and other cancers.

P52

Radiotherapy fraction size sensitivity is modulated by DNA repair systems

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Introduction There is level I evidence that breast cancers are significantly more sensitive to fraction size than previously thought, so that small fractions spare the cancer as much as the dose-limiting normal tissues. In skin, sensitivity to fraction size is associated with the proliferative status of cells in the basal epidermis, which are more sensitive to fraction size during the first 3 weeks of radiotherapy (RT) than during weeks 4 and 5. This study exploits RT-induced proliferation in human epidermis as a model of human cancer to investigate changes in DNA double-strand break (DSB) repair pathways postulated to determine RT fractionation sensitivity.
Methods Thirty patients prescribed 50 Gy/25 fractions over 5 weeks to the breast after tumour excision of early breast cancer were recruited. Then 4 mm punch biopsies of breast skin were collected 2 hours after the first fraction from irradiated and contralateral breast, and 2 hours after the fifth fraction and 1 hour before and 2 hours after the final fraction from irradiated breast. Formalin-fixed paraffin-embedded sections of epidermis were co-stained for β_1-integrin (epidermal stem cells), Ki67 (proliferation), 53BP1 (DSBs), RAD51 (homologous recombination), cyclin A (S–G_2 phase) and p21 (cell cycle arrest).
Results The population of β_1-integrin$^+$Ki67$^+$ cells shows a drop from baseline by day 5 of RT, followed by a significant increase by day 33 ($P = 0.001$). All epidermal cells show 53BP1 foci following RT, but RAD51 foci are present only in a subset of Ki67-expressing cells. Between days 1 and 33, there is a fourfold increase ($P = 0.001$) in the fraction of Ki67$^+$ cells carrying RAD51 foci in the basal epidermis. This correlates with the observation that more basal cells are in the S/G_2 phase of the cell cycle by week 5.
Conclusions Accelerated proliferation in the epidermis at the end of a 5-week course of fractionated RT is associated with an increased adoption of homologous recombination for repairing DSBs. Adoption of homologous recombination, with its high fidelity, offers a mechanism explaining loss of fractionation sensitivity in rapidly proliferating normal (and malignant) cells.

P53

Opticin: a potent anti-angiogenic/antiproliferative agent for breast cancer therapy

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Introduction Opticin, a novel extracellular matrix glycoprotein, is a major component of the vitreous humour of the eye. The vitreous humour is one of the few tissues in the body that is avascular and virtually acellular, and previous studies have indicated that opticin contributes to the maintenance of this state by inhibition of angiogenesis. The aim of this present study is to investigate the effect and mode of action of opticin in suppressing tumour cell proliferation and migration *in vitro* in a panel of breast cancer cell lines and to establish its therapeutic efficacy in human breast tumour xenografts *in vivo*.
Methods A replication defective adenoviral vector constitutively expressing human opticin was generated. A panel of breast cancer cell lines were infected with increasing viral doses and effects on cell proliferation and migration were investigated. Inhibition of proliferation was seen in three out of four of the cell lines used in a dose-dependent manner. In MDA-MB-231 cells, increased apoptosis in the virus-treated cells was observed compared with controls. Virally delivered opticin also reduced migration in all the cell lines studied, again in a dose-dependent manner.
Results To determine whether opticin directly inhibited tumour cell growth *in vivo*, MDA-MB-231 and MDA-MB-468 cells were implanted intradermally into nude mice and tumours allowed to grow to 200 mm before administration of opticin (10^8 plaque-forming units). Vessel density was measured by CD31 immunohistochemistry and a reduction of up to 38% was seen compared with untreated controls. A loss of vessel clarity was also observed. Adenoviral opticin-treated tumours were also significantly smaller than controls.
Conclusions These results demonstrate that opticin has potent anti-angiogenic and anti-proliferative properties both *in vitro* and *in vivo*. Together, the current information demonstrates opticin could be considered a novel cancer therapeutic in the treatment of solid tumours.

P54

Completion of 5-year adjuvant endocrine therapy in the community

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Introduction Adjuvant endocrine therapy is recommended for all eligible patients with breast cancer for a minimum 5-year period. Durations of therapy for a shorter period have worse outcomes for patients. This study examined whether women in the community completed the full course of adjuvant endocrine therapy.
Methods All women diagnosed and treated for breast cancer in the Tayside region of Scotland during the period January 1993 to December 2008 were identified. Information on dispensed prescribing was linked to hospital discharge records, breast cancer clinical audit, cancer registry and death certification for each individual. Patients were identified as starting either aromatase inhibitor (AI) or tamoxifen therapy and their dispensed prescribing analysed to see whether they completed a 5-year course.
Results A total of 5,729 women were identified with an incident cancer within the study. Seventy-one per cent were initially prescribed tamoxifen, 8% AIs and 21% received no adjuvant medication. Nineteen per cent of women started on tamoxifen switched treatments, compared with 25% starting AIs. For patients

followed for at least 1 year, 51 (11%) started on AIs had stopped medication compared with 373 (9%) on tamoxifen. At year 2, an additional 40 (12%) stopped AIs and 384 (11%) tamoxifen. For 3-year follow-up, an additional 39 (19%) stopped AIs, while 351 (11%) stopped tamoxifen. For patients followed for at least 4 and 5 years, respectively, an additional 22 (26%) then 5 (20%) stopped AIs compared with 297 (11%) and 316 (16%) patients who stopped tamoxifen.

Conclusions Large numbers of patients prescribed adjuvant endocrine therapy stop medication before completing the recommended 5-year period, with approximately 20% stopping in the first 2 years. This is likely to have a detrimental effect on patient outcomes. All patients need to be encouraged to continue their medication for the full 5-year recommended period to ensure they receive the maximum benefit.

P55

Psychosocial impact of breast cancer diagnosis and treatment in African, Caribbean and South Asian women

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Introduction Breast cancer is one of the most common forms of cancers in the UK and affects women of all ethnic groups. The psychosocial impact of breast cancer has been well documented. However, the research conducted in this area has been primarily focused on mainstream Caucasian women. There is very little work within the breast cancer literature that captures the experiences of Black and Minority Ethnic (BME) women.

Objective To explore the experiences of breast cancer diagnosis and treatment in African, Caribbean and South Asian women in the UK.

Methods Twenty-three English-speaking breast cancer survivors (11 South Asian and 12 Black women) were recruited for this study. The women were obtained via snowball sampling and through various cancer-related support groups (chain referral sampling). A semi-structured interview was conducted with each participant. The interviews were then transcribed verbatim and inductive thematic analysis was conducted.

Results Thematic analysis of the data revealed six key themes: dealing with the illness as a family, healthcare experiences, body image concerns, social support, spirituality and life post cancer. Support and spiritual beliefs were identified as highly important coping mechanisms.

Conclusions While BME women share similar concerns to Caucasian women, their experiences are also influenced by cultural-specific concerns. This study has important implications for healthcare professionals and recognises the need to provide culturally sensitive care and support to BME women, which is tailored specifically to their cultural values and beliefs.

P56

Intensity and features of acute postoperative pain after mastectomy and breast-conserving surgery

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Introduction Intensity of acute postoperative pain is a known risk factor for the development of chronic postsurgical pain; severe acute pain independently predicts chronic pain up to 1 year after breast cancer surgery [1]. Most studies capture acute pain intensity using numerical or verbal rating scales (NRS/VRS). The objective of this study was to investigate intensity and characteristics of acute postoperative pain, using NRS and verbal descriptors, in the first week after breast surgery.

Methods A prospective cohort study recruiting from four units in north Scotland. The sample was newly diagnosed women with histologically proven primary invasive or non-invasive breast cancer requiring mastectomy or wide local excision (WLE) with/without axillary clearance or sentinel lymph node biopsy. Pain was assessed in the first postoperative week: mean NRS scores at rest and movement; severe pain was defined as NRS >5 [1]. Symptoms of ache, discomfort, pain, numbness and altered sensations were recorded.

Results Of 102 patients, mean age 60.5 years (SD 9.7), one-third (n = 34) had mastectomy and the remainder had WLE. All had axillary surgery: clearance/sample/sentinel lymph node biopsy. Mean NRS scores at rest after mastectomy and WLE, respectively, were: 1.25 (SD 0.4) vs. 1.15 (SD 0.36) (P = 0.23); scores on movement: 1.41 (SD 0.49) vs. 1.15 (SD 0.36) (P = 0.006). Forty-one per cent reported severe pain on movement after mastectomy vs.15% after WLE (P = 0.01). Twenty-two per cent of women reported altered sensations and numbness, mostly in the axilla region.

Conclusions Although mean pain scores were low after surgery, almost one-quarter of patients reported postoperative numbness or altered sensations. Studies of postoperative pain should include assessment of pain character in addition to pain intensity.

Reference

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P57

Breast cancer knowledge among women with learning disabilities and their experiences of breast mammography

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Introduction As the life expectancy of people with learning disabilities rises, so too do cancer prevalence rates in people with learning disabilities. Despite the efforts of government policies to ensure equal access to improve health screening, the uptake for breast mammography within this population still remains lower than that of the general population. The purpose of this study was to ascertain the knowledge of breast cancer among women with a learning disability and to explore their experiences of breast mammography.

Methods A qualitative approach using four focus groups with women with learning disabilities was employed, and a semi-structured interview schedule aided the process.

Results Associated risks, preventative factors and signs and symptoms of breast cancer were extremely limited with their sources of knowledge primarily coming from carers or nursing staff. Positive attitudes towards breast mammography were reported; however, these women also described negative feelings of fear and anxiety, attributed to a lack of understanding about the screening process. Emotional support and information were seen to reduce negative feelings. A lack of information and embarrassment were identified as the main barriers to screening. Ongoing support from others such as family members and carers, accessible information and health promotion and education were considered to be main solutions for encouraging attendance for breast mammography.

Conclusions This study highlights the need for health promotion and education for women with a learning disability, their family carers and staff working with this target group in order to enhance the knowledge and awareness of breast cancer and screening. This not only will aid in reducing the adverse affects of breast mammography but will ensure that informed decisions about breast screening are made. More accessible multiformat information for women with a learning disability is essential in order to facilitate health promotion and education.

P58

A TRAIL-R1-specific ligand in combination with doxorubicin selectively targets primary breast tumour cells for apoptosis

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Introduction Although the majority of tumour cell lines, including breast cancer cell lines, are sensitive to the death-inducing ligand and potential cancer biotherapeutic TNF-related apoptosis-inducing ligand (TRAIL), most primary tumours are TRAIL-resistant. Importantly, doxorubicin, a chemotherapeutic agent commonly used in breast cancer, has previously been shown to sensitize TRAIL-resistant breast cancer cell lines to TRAIL. Furthermore, using receptor-selective ligands (patent filed by MRC Technology) specific for the TRAIL death receptors, TRAIL-R1/TRAIL-R2, we have previously shown that primary leukaemic cells isolated from patients with chronic lymphocytic leukaemia can be selectively sensitized to apoptosis by combining an a histone deacetylase inhibitor (HDACi) with a TRAIL-R1-specific form of TRAIL/TRAIL-R1 mAb.

Methods and results To examine the potency of TRAIL-R1/TRAIL-R2-specific ligands in breast cancer, a panel of breast tumour cell lines was employed, which

included the TRAIL-resistant breast cancer cell line, T47D. In addition, a modified approach of culturing primary breast tumour explants *ex vivo* to maintain their three-dimensional architecture provided a more clinically relevant breast tumour model. Importantly, all TRAIL-sensitive breast tumour cell lines responded *only* to a TRAIL-R1-specific form of TRAIL. Despite expressing TRAIL-R1/TRAIL-R2, the T47D cell line required initial sensitization by doxorubicin and again exhibited selectivity towards apoptosis induced by a TRAIL-R1-selective ligand. Crucially, we show that doxorubicin can also sensitize TRAIL-resistant primary breast tumour explants to TRAIL-induced apoptosis, while having no effect on normal breast tissue. Furthermore, in this *ex vivo* model, TRAIL combined with doxorubicin induced significantly more apoptosis via TRAIL-R1 than TRAIL-R2.

Conclusions Our results have important implications for the potential treatment of breast cancer with TRAIL-based therapeutic agents. We propose that using a TRAIL-R1-specific ligand/mAb combined with subtoxic concentrations of doxorubicin will selectively target tumour cells and minimise potential side effects, such as triggering of TRAIL-induced prosurvival pathways in TRAIL-resistant primary tumour cells or cardiotoxicity induced by higher concentrations of doxorubicin used in monotherapy.

P59

Modelling breast cancer in a three-dimensional heterotypic culture system

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Microenvironmental factors are fundamental in the regulation of normal and tumour breast tissue. Two cell types have been implicated in having opposing effects on breast tumour cell behaviour: myoepithelial cells exhibit broad tumour-suppressor activity, whilst fibroblasts frequently promote tumour growth and invasion. Previous work has described the development of a physiologically relevant three-dimensional heterotypic culture system containing tumour, myoepithelial and fibroblast cells. The data showed organisation of the cells into co-unit structures recapitulating ductal carcinoma *in situ* breast, with homing of myoepithelial cells around luminal cells, and highlighted a central role for tumour-associated fibroblasts in disrupting ductal carcinoma *in situ* structures. This study describes further manipulation of the model to include tumour cells that represent the heterogeneity of breast cancer.

MCF-7 (ER^+), MDA-MB-468, MDA-MB-231 (basal) and MDA-MB-453 ($Her2^+$) were cultured in collagen for 7 days in the presence or absence of normal myoepithelial cells. Gels were fixed in formalin, paraffin embedded and immunohistochemistry was performed for a series of markers recognising the cell types along with basal polarity and basement membrane proteins.

Initial morphological analysis of the cultures has been performed to assess the degree of co-unit formation, based on a visual description of the size and shape of the co-units. Co-unit formation has been employed as a representative measure of tumour progression as it is known to be a key feature in early breast cancer invasion. When cultured alone, MCF-7 and MDA-MB-468 cells formed spherical co-unit structures and this was maintained in the presence of myoepithelial cells. In contrast, MDA-MB-231 and MDA-MB-453 cells show a more scattered appearance. The presence of myoepithelial cells induced polarity in the MDA-MB-231 cells and a more ordered appearance.

This study is the first time that the co-culture of tumour cell populations with myoepithelial cells has been investigated in three-dimensional collagen gels showing differences in morphology that may relate to tumour progression.