7C BREAST SURGERY 2

O169 THE CELL ADHESION PROTEIN JAM-A IS REQUIRED FOR MAINTENANCE OF TUMOUR DORMANCY IN BREAST CANCER CELLS

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Introduction: Dormant cancer cells within bone marrow interacting with stromal cells promote survival of breast cancer micro-metastases. Tumour dormancy can be modelled in vitro by exposing cancer cells to bFGF-2, a mammary differentiation factor abundant in the bone marrow stroma which causes partial re-differentiation, cell spreading and re-expression of integrin-α5β1. Ligation of integrin-α5β1 by fibronectin and activation of the PI3K pathway both contribute to dormant cell survival. Our study has focussed on the potential role in tumour dormancy of JAM-A, a cell adhesion protein whose overexpression has been linked to increased risk of metastasis. Since JAM-A is required for the angiogenic functions of bFGF-2 and loss of JAM-A causes down-regulation of the α5β1 downstream effector FAK, we hypothesized that JAM-A is required for maintenance of breast cancer dormancy.
Method: The role of JAM-A in creating or maintaining dormant phenotypes in cultured breast cancer cells was examined by gene silencing, immunofluorescence and western blotting.
Result: MCF-7 breast cancer cells silenced for JAM-A had reduced α5β1-integrin expression and failed to form a dormant phenotype on fibronectin-coated plates in the presence of bFGF2. In addition they retained metabolic activity, and down-regulation of phospho-FAK was observed compared to JAM-high dormant clones.
Conclusion: JAM-A is required for maintenance of dormancy in breast cancer cells in a simulated bone marrow microenvironment, and represents a novel therapeutic target worthy of investigation in breast cancer. Abbreviations: bFGF-2- Basic Fibroblast growth factor JAM-A Junctional adhesion molecule FAK- Focal adhesion kinase
Take-home message:
JAM-A is required for maintenance of dormancy by breast cancer cells and it could be novel therapeutic target worthy of investigation

O170 WITHDRAWN

O171 S100B AS A SERUM BIOMARKER IN ENDOCRINE RESISTANT BREAST CANCER

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Introduction: In estrogen receptor positive breast cancer, endocrine therapy is the standard line of treatment and even though it results in reduced recurrence and mortality, a significant number of patients will eventually relapse. Early detection of metastatic disease would significantly enhance management of endocrine resistant breast cancer.
Method: The expression of S100β in tissue and serum was assessed by immunohistochemistry of tissue and a ELISA based clinical assay for serum in two retrospective cohorts of ER-positive endocrine treated breast cancer patients. Pre-operative and post-operative matched serum samples and sequential post-operative levels of S100β serum was also assessed.
Result: Primary tumor tissue expression of S100β protein in a retrospective cohort of 509 endocrine treated breast cancer patients indicated a significant reduction in time to disease recurrence (HR. 2.32, 95% C.I. is 1.545 to 3.40, $p<0.0001$). S100β protein is also detectable in serum of breast cancer patients and elevated levels of serum S100β prior to removal of primary tumor is associated with poor disease free survival in endocrine treated patients (n=187, HR. 3.58, 95% C.I. is 1.59 to 9.39, $p=0.003$). Serum levels of S100β are significantly reduced after primary tumor resection (n=55, $p=0.023$). In serial samples taken during the treatment period, elevated levels of S100β significantly associated with disease progression and with the emergence of metastatic disease ($p=0.019$).
Conclusion: Associations between elevated levels of serum S100β and subsequent disease progression in endocrine treated patients, suggests S100β as a monitoring tool for identifying ER-positive breast cancer patients at high risk of relapse.
Take-home message:
S100B as a new potential serum biomarker in endocrine resistant breast cancer.

O172 JUNCTIONAL ADHESION MOLECULE-A (JAM-A) AS A NOVEL THERAPEUTIC TARGET IN DUCTAL CARCINOMA IN SITU (DCIS)

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Introduction: Junctional Adhesion Molecule-A (JAM-A) is a cell-cell adhesion protein whose increased expression is associated with poor prognosis in patients with invasive breast cancer. However little is known about its potential role in earlier forms of breast cancer, specifically ductal carcinoma in situ (DCIS). We investigated whether JAM-A is also overexpressed in DCIS, and whether it may represent a novel therapeutic target in early breast cancer.

Method: A tissue microarray of DCIS cases was immunohistochemically stained for JAM-A expression and semi-quantitatively scored. A small molecule inhibitor of JAM-A (JBS-2), was designed and tested in a HER2/JAM-positive cell line model of DCIS, SUM-225. Cell proliferation was assessed following treatment with a HER2/EGFR tyrosine kinase inhibitor (lapatinib) or JBS-2.
Result: A commercial Tissue Microarray comprising of DCIS (n=50) and normal adjacent tissue (NAT) (n=26) indicated that 96% of patients with DCIS had moderate/high JAM-A expression in comparison to 24% of NAT cases. Treatment of SUM-225 cells with JBS-2 or lapatinib significantly inhibited cellular proliferation in a concentration-dependent manner. This suggests that SUM-225 cells may be a useful model in which to examine pharmacological co-targeting of JAM-A, HER2 and/or EGFR.
Conclusion: The role of JAM-A in early stage breast cancer is largely unknown. Increased expression of JAM-A in DCIS coupled with the responsiveness of a DCIS cell line model to a JAM-A inhibitor suggests value in investigating JAM-A inhibitors as preventative agents in this setting. JAM-A inhibitors may also elicit additive anti-tumorigenic effects in combination with HER2 inhibitors in HER2 positive DCIS.
Take-home message:
Junctional Adhesion Molecule-A (JAM-A) may represent a novel therapeutic target in early stage breast cancer such as Ductal Carcinoma in situ and suggests value in investigating JAM-A specific inhibitors as preventative agents to limit invasive disease progression.

O173 RESPONSE OF BREAST CANCER CELLS TO 1.9NM GOLD NANOPARTICLES

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Introduction: Radiotherapy is a core treatment for many cancers and inclusion of Gold Nanoparticles (GNP) has the potential to increase efficacy (Dose Enhancement; DE). Previously, by clonogenicity assay, we observed that 1.9nm GNP increased cell killing following 6MV irradiation. To investigate cell and molecular changes, studies were repeated and compared with Doxorubicin (DOX) treatment both in 2D and 3D.
Method: In 0.5% stripped serum, MDA-MB-231 and MCF-7 breast cancer cells were exposed to: 0.25–1mg/mL GNP or 1nM-10μM Doxorubicin for 24hrs, or left untreated, then exposed to 0-6Gy X-irradiation (XR). Cell death was monitored at 24hrs and 48hrs by LDH release; metabolic activity at 48hrs was measured by MTT assay. Penetration of GNP in 3D spheroids was investigated by TEM.
Result: In MB-231 cells, GNP (0.25-1mg/mL) caused significant cell death ($p<0.0001$) by 48hrs, which was not enhanced by XR. In contrast, XR (1.5-6Gy) significantly enhanced 5μM and 10μM DOX induced cell death ($p<0.0001$) within 24hrs. 1.9nm GNP were taken up by peripheral cells of the spheroid to a depth ~130μm (approximately 40% of the volume) and localized predominantly within the lysosomes. MCF-7 cells were more sensitive to GNP and XR: GNP (0.25-1mg/mL) caused significant cell death within 24hrs ($p<0.001$) which was enhanced by XR ($p<0.01$ – p0.001).
Conclusion: 1.9nm GNP alone induced significant cell death, which appeared unlikely to result from direct DNA-damage; on the other hand, Doxorubicin remains a powerful DNA-damaging agent. The differential responses reflect the heterogeneity of the breast cancer cells.
Take-home message:
Gold Nanoparticles induce significant cell death appearing unlikely from direct DNA-damage, but Doxorubicin remains to be a powerful DNA-damaging agent.

O174 MEK INHIBITION: A FUTURE BREAST CANCER THERAPEUTIC?

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Introduction: Hyperactivation of the MAP-kinase pathway is a critical development in many cancers, especially as it interacts with numerous signalling pathways. The kinases: MEK1/MEK2 are suitable targets for intervention, which is reflected by the number of clinical trials currently underway. Whether MEK inhibition (MEKI) is a potential therapeutic for breast cancers was investigated.
Method: In 2D cell culture: activation and inhibition of the MAPK pathway (phosphorylation of ERK1/ERK2) was investigated in a panel of breast cancer cell lines by Western Blot (WB); the cytotoxic and metabolic effects of MEKI were investigated by LDH release and MTT activity. Responses to the MEK inhibitor AZD6244 were observed in 3D cultures of ZR-75-1 and MDA-MB-231 cells. Expression levels of receptors and DUSP4 were determined by WB and qRT-PCR.
Result: In 2D: AZD6244 significantly inhibited proliferation of MCF-7, ZR-75-1, T47D and MDA-MB-231 cells ($p<0.0001$) and U0126 significantly inhibited MCF-7, T47D, MB-231 and ZR-75-30 cells ($p<0.0001$). Notably, oestrogen stimulation counteracted any MEK inhibition of ER-positive cells. In 3D: at 1M and 10M AZD6244 prevented cell proliferation and formation of MDA-MB-231 spheroids, while 100nM, 1M and 10M inhibited the growth of ZR-75-1 spheroids ($p<0.005$ – $p<0.0001$). DUSP4 was expressed at variable levels in all cell lines examined (10).
Conclusion: MEKI may have a therapeutic role in certain breast cancers; here, responses to different inhibitors were variable and reflected cancer cell heterogeneity. Positive responses to MEKI did not correlate with KRAS or BRAF mutations or to loss of DUSP4 expression.

Take-home message:
MEK inhibitors may have a therapeutic role in certain breast cancers. Variable responses to different inhibitors reflect the heterogeneity of cancer cells.

O175 ADAM22 AS A PREDICTIVE MARKER FOR ENDOCRINE RESISTANCE WITH A COMPANION THERAPEUTIC

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Introduction: Endocrine resistance is a major hurdle in the fight against ER+ breast cancer. In the 30% of patients that recur, a significant proportion will overexpress the ER co-activator SRC-1, which mediates metastatic development. Recently, ADAM22 was shown to be regulated by SRC-1 in the resistant setting. ADAM22 plays roles in both migration and de-differentiation of tumours, while in a large breast cancer cohort, expression was shown to predict poor disease free survival. Finally, treatment with the ADAM22 ligand, LGI1, alleviated this aggressive phenotype in vitro. This work aims to elucidate ADAM22 functions and LGI1 effects in endocrine resistance.
Method: ADAM22 function was assessed in the resistant setting in vitro, through functional assays and high throughput reverse phase protein array (RPPA). A small LGI1 peptide mimetic was designed in silico. Efficacy of the LGI1 mimetic was evaluated in vitro and in vivo. Potential off target effects of ADAM22 targeting was determined by immunohistochemical staining of healthy organs.
Result: Functionally, ADAM22 is important for the development of anchorage independent colonies. RPPA analysis suggests an anti-apoptotic role for ADAM22. The LGI1 mimetic inhibits migration and colony formation in vitro, while preliminary in vivo studies indicate inhibition of tumour growth and metastases. IHC studies show ADAM22 expression is limited to the brain.
Conclusion: ADAM22 is a key player in endocrine resistant metastatic development. Through inhibition of apoptosis, ADAM22 may confer survival of tumour cells. This can be partially overcome via LGI1 mimetic treatment and ADAM22 targeting should yield relatively low off target effects.
Take-home message:
ADAM22 is a promising predictive marker for endocrine resistant metastatic breast cancer.

O176 DEAD END IN TREATING TAMOXIFEN RESISTANT BREAST CANCER? USING FASLODEX TO OVERCOME TAMOXIFEN RESISTANCE IN ER-POSITIVE BREAST CANCER

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Introduction: Breast cancer is one of the most commonly diagnosed cancers in women worldwide. 70% of breast cancers are ER (estrogen receptor)-positive, for which endocrine therapies such as tamoxifen are a mainstay treatment. Unfortunately, 40% of patients relapse and stop responding to therapy, leading to disease progression. This lab has previously developed tamoxifen-resistant cells that grow metastatic tumours in animal models in the presence of tamoxifen. Clinically it is important to identify the mechanisms of resistance and to identify alternative druggable targets. The aim of this study was to determine if alternative targeted therapies such as Faslodex can be used to overcome tamoxifen resistance in the ER-positive model of breast cancer.

Method: This study utilised tamoxifen-resistant cell lines, LY2 and TamR. MTS proliferation assay and immunohistochemistry was carried out to measure the effect of fasoldex and other therapies on cell proliferation. ER expression was monitored in the presence and absence of Faslodex using western blots.
Result and **Conclusion** Faslodex had no significant effect on cell proliferation (n=3, p=n.s.), despite reducing ER expression in tamoxifen-resistant breast cancer cells. Even though estrogen signalling was successfully disrupted in these cells, no reduction in cell proliferation was observed as a consequence. These results suggest that the tamoxifen-resistant breast cancer cells may not be dependent on estrogen signalling. Finally, we show that targeting methylation, a mechanism implicated in endocrine-resistance, may be a viable alternative as it showed a marked reduction in proliferation. In conclusion, to overcome resistance, alternative therapies targeting methylation pathways need to be explored.
Take-home message:
Our study shows that faslodex does not overcome tamoxifen resistance in ER-positive breast cancer cells. Therefore, alternative therapies targeting methylation pathways need to be explored.

O39 SIGNIFICANT VARIATIONS IN ELDERLY BREAST CANCER MANAGEMENT WITHIN A SINGLE CITY: CAN LEVELS OF COMORBIDITY PROVIDE AN EXPLANATION?

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Introduction: Previously we compared the management of elderly breast cancer patients in two neighbouring hospitals within a UK city, and found Unit 1 to operate on a significantly higher proportion of patients, and to treat fewer with endocrine therapy alone, compared to Unit 2. No difference in age, tumour pathology or deprivation scores was found. We have evaluated levels of comorbidity as a possible explanation.
Method: Breast cancer patients, aged over 70 years, treated at two hospitals between 2009 and 2013, were identified from a database collected prospectively within the regional Managed Cancer Network.

Comorbidity data was gathered from the electronic clinical record and used to calculate the Charlson Comorbidity Index. Scores were compared using Chi square test for linear association.
Result: 954 elderly breast cancer patients were treated in the two units with similar numbers in each. Patients treated with hormone treatment only had a significantly higher Charlson score (mean 5, range 3-12) compared to patients with surgical treatment (mean 4, range 3-13) (p=0). 15.4% patients operated on at Unit 1, compared to 11.0% at Unit 2, had Charlson score 6-9, and 0.8% surgical patients at Unit 1 had score of 10+ compared to 0% in Unit 2 (p=0.036).
Conclusion: Our findings suggest that Unit 1 operated on patients with higher levels of comorbidity than Unit 2. Further work is warranted to ascertain multidisciplinary team members attitudes and preferences at each site to explain the significant difference in management in two neighbouring units.
Take-home message:
There is significant variation in management of elderly patients with breast cancer which can in part be attributed to differences in attitudes towards operating on patients with high levels of comorbidity between different hospitals.