O1    NEOADJUVANT CHEMOTHERAPY INDUCES CHANGES IN EXPRESSION OF BREAST CANCER RESISTANCE PROTEIN THAT PREDICT DISEASE FREE SURVIVAL IN BREAST CANCER

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Introduction
Three main xenobiotic efflux pumps have been implicated in modulating breast cancer response to chemotherapy. These are Pgp (P-glycoprotein, multidrug resistance protein-1), MRP1 (multidrug resistance-associated protein 1), and BCRP (breast cancer resistance protein). We aimed to investigate expression of these proteins before and after neoadjuvant chemotherapy (NAC) to determine whether levels define response to NAC or subsequent survival.

Methods
Paraffin embedded tissue was collected representing matched pairs of core (pre-NAC) and surgical specimens (post-NAC) from 45 patients with invasive ductal carcinomas (follow-up range 3-8.8 years). NAC regime consisted of anthracyclines +/- taxanes. Immunohistochemistry was performed. A computer-aided scoring protocol was developed and validated against 2 independent observers (intra-class correlation coefficient 0.83 and 0.82).

Results
Pgp and MRP1 expressions were significantly upregulated after exposure to NAC (p=0.0024 and p<0.0001). BCRP expression showed more variation in response to NAC: individual cases showed either down-(41%) or up-regulation (59%) after NAC. Pre- or post-NAC expression of Pgp, MRP1 or BCRP did not correlate with response to NAC or survival. However, differences in expression of BCRP between pre- and post-NAC samples correlated with disease free survival (DFS): significantly longer 5-year DFS was seen in patients where down-regulation occurred compared to up-regulation (80% vs. 40%; Log rank p=0.007).

Conclusion
BCRP up-regulation after exposure to NAC is an adverse event, and investigating mechanisms to inhibit this process may improve NAC efficacy.

Take-home message
Significantly shorter disease free survival was seen in patients where BCRP up-regulation occurred after exposure to NAC. Investigation of mechanisms to inhibit BCRP up-regulation may improve NAC efficacy in breast cancer.

O2    THE SIGNIFICANCE OF OESTROGEN RECEPTORS IN OESOPHAGEAL CANCER DEVELOPMENT

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Introduction
A recent nested case-control study within a UK cohort and meta-analyses suggested that the use of menopausal hormone therapy has been associated with reduced risk of oesophageal cancer (OC). In this study, the potential significance of oestrogen and its receptors (ER) as therapeutic biomarkers in OC is investigated.

Methods
Following local REC approval, the OC cell lines, OE33 and OE19, were used to study the effect of ER modulators on cell proliferation using 5-bromo-2’-deoxyuridine (colorimetric) assay. ERα and ERβ expression profiling in paired normal oesophageal mucosa and tumour tissues (n = 34) was performed using quantitative Real Time Polymerase Chain Reaction (RT-PCR). Correlation between expression levels of ER with the clinico-pathological features for OC was also determined.

Results
There was a significant dose dependant inhibition of proliferation in OE33 and OE19 OC cell lines by a highly selective ERα antagonist (MPP) and ERβ specific antagonist (PHTPP) (p <0.05). RT-PCR analysis revealed that ERα and ERβ mRNA expression was significantly higher (p<0.05) in tumour tissues relative to their paired normal mucosa. Moreover, expression of ERα and ERβ in the tumour tissue samples correlated inversely with survival outcome (p<0.05). Finally, up-regulation of ERα correlated with higher pathological T stage (p<0.05).
and lymph node metastasis (p<0.05), while ERβ up-regulation correlated with positive vascular invasion (p<0.05).

Conclusions
There is a significant up-regulation of ER mRNA in OC which has an adverse prognostic correlation. The inhibition of OC cell lines by highly selective ER modulators indicates that ER may have a potential therapeutic significance in the treatment of OC.

Take-home message
In this study, it has been demonstrated that up-regulation of ER has an unfavourable relationship with survival and pathological features of OC. Furthermore, selective blocking of ER seems to inhibit OC cell proliferation. Therefore, ER system may provide an additional novel target for the treatment of OC.

O3 AN OMEGA-3 RICH LIPID EMULSION IS ASSOCIATED WITH IMPROVED CLINICAL OUTCOME IN PATIENTS WITH SEVERE ACUTE PANCREATITIS: A RANDOMISED DOUBLE-BLIND CONTROLLED TRIAL
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Introduction
Early deaths in patients with severe acute pancreatitis (SAP) are usually caused by multiple organ failure driven by acute inflammation. Omega-3 fish oil (n-3 FO) has proven anti-inflammatory properties. We aimed to investigate the effects of n-3 FO in SAP patients.

Methods
We conducted a randomised double-blind controlled trial in patients with SAP. The two groups received a lipid emulsion with (n-3 FO group) or without (control group) fish oil in conjunction with standard care. Infusions were initiated within 72 hours of symptom onset and for a maximum of 7 days. Organ failure, ITU/HDU admission, length of stay, septic complications and mortality were recorded.

Results
Fouaty-four patients were included with equal numbers (22) in each group. 19/22(86%) of the control group and 14/22(64%) patients in the n-3 FO group developed one or more organ failure with 13/22 (59%) of the control group and 6/22(27%) of the n-3 FO group developing new organ failure (p=0.033). Eleven patients (50%) in the control group vs 5/22(23%) in the n-3 FO group required admission to the ITU/HDU, p=0.060. The fish-oil group had significantly shorter ITU/HDU and hospital stay (11+/3 vs 7+/11, p=0.029 and 12+/6 vs 21+/13, p=0.043, respectively). Septic complications occurred in 8(36%) patients of the fish-oil group and 11(50%) of the non-fish oil group, p=0.361. There were 2 deaths (1 in each group).

Conclusion
Fish oil based lipid emulsion is associated with fewer organ failures; better recovery and shorter ITU/HDU and hospital stay in patients with SAP. A larger multicentre trial is warranted.

Take-home message
Fish oil emulsions improve the clinical outcome in patients with severe acute pancreatitis.

O4 IDENTIFICATION OF IL8 AND TIMP4 AS MOLECULAR PHENOTYPIC DESCRIPTORS OF BREAST CAPSULAR CONTRACTURE FORMATION USING INFORMATICS ANALYSIS OF THE WHOLE GENOME TRANSCRIPTOME
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Introduction
Breast capsular contracture formation following silicone implant augmentation/reconstruction is a common complication that remains poorly understood. Several candidate genes have been associated with this condition; however, there are no published studies to date that have utilised whole genome microarrays in identifying biomarkers. The aim of this study was to identify potential biomarkers implicated in breast capsular contracture formation by using whole genome arrays.

Methods
Biopsy samples were taken from 18 patients (23 breasts) with Baker Grade I and II (classified as mild contracture) and Baker Grade III and IV (classified as severe contracture). Whole genome microarray was
performed and 6 significantly dysregulated genes were identified for validation with quantitative reverse transcriptase polymerase chain reaction (QRT-PCR). Haematoxylin and eosin (H&E) and quantitative immunohistochemistry (IHC) analysis was then performed on the breast capsules.

**Results**

Microarray results showed that the genes ACAN, TIMP4, and TNFSF11 were significantly downregulated in severe contracture; while, MMP12, SAA1, and IL8 were significantly upregulated when compared to mild contracture. The dysregulation of ACAN, TNFSF11, TIMP4 and IL8 was validated by QRT-PCR. (p values <0.05).

Quantitative IHC showed increased expression of IL8 and MMP12 in severely contracted capsules (p <0.05), and decreased expression of TIMP4. No statistical significance was found between Baker grades for ACAN, TNFSF11 and SAA1.

**Conclusion**

For the first time, this study has shown a number of unique biomarkers of significance in capsular contracture formation. IL8 and TIMP4 may serve as potential key diagnostic, therapeutic and prognostic biomarkers in capsular contracture formation.

**Take-home message**

Breast capsular contracture formation is the most common complication post breast augmentation/reconstruction and its pathoetiology is still not clearly understood. This paper describes the results of the first whole genome microarray ever performed on breast capsules, verified with QRT-PCR and quantitative IHC and H&E, which found IL8 and TIMP4 as two potential key diagnostic, therapeutic and prognostic biomarkers in capsular contracture formation.

**O5 AVAILABILITY OF T CELL HELP DETERMINES ALLOANTIBODY LEVELS AND GRAFT OUTCOME IN A MURINE MODEL OF ANTIBODY-MEDIATED REJECTION**

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**Introduction**

The mechanisms by which alloantibody affects acute or chronic allograft rejection remain unclear. Here we hypothesise that the availability of T cell help determines the outcome of antibody-mediated rejection.

**Methods**

MHC-mismatched BALB/c hearts were transplanted into T cell-deficient (TCR-/-) CB57BL/6 recipients. T cell help was provided by transfer of either high (10^5 cells) or low (10^3 cells) numbers of TCR-transgenic TCR75 CD4 T-cells that recognise Class I donor H2-Kd antigen as self-restricted processed peptide via the indirect pathway. Alloantibody production was determined by anti-H-2Kd ELISA. CB57BL/6 Rag2/- recipients that lack both T and B cells were used as controls.

**Results**

Reconstitution of TCR-/- recipients of BALB/c hearts with 10^5 TCR75 T cells resulted in strong alloantibody responses and acute graft rejection (MST 9 days, n=9). Reconstitution with 10^3 CD4 T cells resulted in much more modest alloantibody production, but was nevertheless associated with endothelial complement deposition, development of progressive allograft vasculopathy and gradual graft loss (MST 50 days, n=6). In contrast, BALB/c hearts survived indefinitely, with minimal allograft vasculopathy, when transplanted into control Rag2/- recipients reconstituted with either high or low numbers of TCR75 T cells. Notably, passive transfer of Rag2/- recipients with immune serum from TCR-/- recipients reconstituted with 10^5 TCR75 T cells resulted in acute heart graft rejection and florid intragraft complement deposition.

**Conclusion**

The magnitude of the alloantibody response determines whether humoral alloimmunity effects acute or chronic rejection, and is in turn determined by the availability of T cell help. (MST – mean survival time)

**Take-home message**

The availability of T cell help is a critical factor in determining the size of the alloantibody response, and its consequent ability to mediate acute or chronic graft damage in a newly-developed model of antibody-mediated rejection.

**O6 IRON CHELATION IN THE TREATMENT OF OESOPHAGEAL ADENOCARCINOMA – IN-VIVO ACTION OF DEFERASIROX ON A XENOGRAFT MODEL**

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**Introduction**

The mechanisms by which iron overload exacerbates tumour growth and metastasis in a range of cancer types have been extensively studied, and novel strategies to reduce iron levels are actively being investigated. Iron chelation therapy has previously been shown to improve outcomes in preclinical models of cancer, with a number of iron chelators having shown promising anti-tumour activity in clinical trials. Deferasirox is a potent iron chelator that is currently being investigated for the treatment of haemochromatosis, but its efficacy against oesophageal adenocarcinoma has not been studied.
The malignant progression of Barrett’s metaplasia to oesophageal adenocarcinoma (OAC) is associated with altered expression and function of the pertinent cellular iron transport proteins. These alterations result in increased cellular iron loading which is likely to drive cellular proliferation; a key hallmark of cancer. This is further supported by a wealth of data demonstrating that iron chelators cause cell cycle arrest and apoptosis. The aim was to determine if the clinically established iron chelator Deferasirox has in-vivo anti-neoplastic activity by utilising a xenograft model of OAC.

**Methods**

A murine model of OAC was produced by xenografting OE33 and OE19 OAC cells subcutaneously into NOD-SCID mice. Deferasirox was gavarged as an oral agent on alternate days for 3 weeks. The mice were culled and the xenograft, liver, spleen, heart and blood harvested for analysis. All animal work was performed under Home Office approved conditions.

**Results**

Oral Deferasirox significantly impaired xenograft growth compared to controls; 43% (p=0.0061) and 42% (p=0.05) average reduction in xenograft weight for OE33 and OE19 respectively. Deferasirox treatment was well tolerated with no change in average mouse weight compared to control. Crucially, iron chelation did not induce systemic anaemia or major organ dysfunction.

**Conclusion**

The clinically established oral iron chelator Deferasirox appears to have significant in-vivo anti-neoplastic effects in a murine xenograft model of OAC. Furthermore, after three weeks of dosing without oral iron supplementation, no systemic signs of anaemia were observed. Oral iron chelators should be considered as anti-neoplastic agents in human trials.

**Take-home message**

Clinically established iron chelators exhibit potent anti-neoplastic effects without systemic iron stripping. Iron chelators should be trialled as an adjunct to standard chemotherapeutic agents in the treatment of oesophageal adenocarcinoma.

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**O7 GENERATION OF HLA-SPECIFIC HUMANISED MICE USING BONE MARROW-DERIVED HAEMATOPOIETIC STEM CELLS FROM CADAVERIC ORGAN DONORS**

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**Introduction**

‘Humanised’ mice reconstituted with a functional immune compartment are an invaluable tool in the study of the immune response to human cells and tissues and can be generated using haematopoietic stem cells (HSCs) from a variety of sources. However, bone marrow (BM) from cadaveric organ donors represents the only potentially abundant source of HSCs of specified HLA type. We therefore examined the ability of BM-derived HSCs from cadaveric organ donors to generate a humanised mouse model to investigate human immune responses to alloantigens.

**Methods**

Bone marrow was aspirated from the lumbar vertebrae and iliac bones of human cadaveric donors after other organs were retrieved for transplantation and the bone marrow was separated using Ficoll gradient and frozen at -80°C. After thawing, live CD34+ HSCs were isolated using magnetic beads and adoptively transferred into sub-lethally irradiated immunodeficient NOD/SCID/IL2ry-/- mice. Engraftment was assessed by flow cytometric analysis of peripheral blood samples at weekly intervals.

**Results**

Bone marrow was successfully harvested from cadaveric donors that proceeded to donation after either brain death or circulatory death (oldest donor aged 78). Post-thaw viability of the CD34+ fraction was routinely >80% and NSG mice were successfully reconstituted with CD45+ human B (70.1%), CD4 (3.2%) and CD8 (2.57%) T lymphocytes by 8 weeks.

**Conclusion**

Our data show that BM-derived HSCs survive circulatory arrest for several hours and maintain their engraftment potential in immunodeficient mice. This model enables the generation of HLA-specific humanised mice, using a readily available source of HSCs, to investigate human immune responses to alloantigens.

**Take-home message**

Bone marrow from cadaveric organ donors is a viable source of haematopoietic stem cells for the generation of humanised mice.