ORAL PRESENTATION 2C
BREAST SURGERY

070 INVESTIGATING THE IMPORTANCE OF VARIANTS AT 12P11, 12Q24 AND 21Q21 IN BREAST CANCER IN THE WEST OF IRELAND
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Introduction
A large genome-wide association study found that novel breast cancer susceptibility loci at 12q24 (rs1292011); 12p11 (rs10771399) and 21q21 (2823093) were associated with breast cancer in women of European ancestry. The aim of our study was to investigate the prevalence of variants at these three loci in a specific Irish subpopulation, and to examine the association between these variants and breast cancer in this cohort.

Methods
Blood samples were collected from patients from the West of Ireland with breast cancer (cases), as well as from healthy female controls. DNA was extracted from these samples using a salting out method. Genotyping was then performed using a custom Taqman assay, and the process repeated for each target. The Hardy-Weinberg test of equilibrium was performed for each target.

Results
A total of 1639 samples were genotyped, including 1191 cases and 448 controls. The variant at 21q21 was found to be out of H-W equilibrium ($X^2$=6.01), and the variant at 12p11 borderline ($X^2$=3.53). Sixty-nine per cent of breast cancer cases demonstrated at least one copy of the variant at 12q24 compared to 72% controls (p=0.426, $X^2$). There was no difference in the prevalence of the variant across molecular subtype, grade or stage.

Conclusion
All three variants were detected in the population in the west of Ireland. However, the observed frequency of two of the variants was far below that expected. This finding reflects genetic heterogeneity even within continents, and highlights the need for population-specific investigation for potential disease-causing variants.

Take-home message
The frequencies of genetic variants vary across populations, and population-specific investigation is required to investigate the role putative disease-causing targets in that particular cohort.

071 THE TISSUE FACTOR PATHWAY MAY PROMOTE BREAST CANCER STEM CELL ACTIVITY
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Introduction
The clotting system is dysregulated in over 50% of cancers and plays an important role in promoting metastasis. The clotting protein Tissue Factor (TF) is implicated in promoting the survival of cancer stem cells (CSC), a subpopulation of cells that can initiate tumours, are able to self-renew and may be responsible for cancer recurrence. We sought to determine TFs role in breast CSC using in vitro assays

Method
In human breast cancer cell lines, CSC activity was measured using non-adherent tissue culture, including the mammosphere assay, and analysis of ALDH1 activity using fluorescence-activated cell sorting (FACS). TF activity was inhibited using transient silencing RNA (siRNA) and stimulated with the TF agonist factor VIIa (FVIIa). Cells were sorted according to TF expression into TF positive and TF negative cells using FACS.

Results
MDAMB-231 and SKBR3 cell lines had high TF and T47D and MCF7 had low TF expression. In cells that survived non-adherent culture (anoikis-resistant), TF expression was higher compared to control in T47D but unchanged in SKBR3 and MCF-7s. When sorted by FACS,
TF-expressing T47D cells had increased mammosphere formation, compared to TF-negative cells. TF siRNA reduced mammosphere formation and ALDH1 activity in MDAMB-231 and T47Ds. FVIIa increased mammosphere formation and ALDH1 activity in MDAMB-231 and T47D cells but not MCF-7s. The effects of FVIIa on mammosphere formation was abrogated by TF silencing in T47Ds.

**Conclusion**
TF-VIIa signalling may play an important role in breast CSC activity and may be a potential target in the treatment of breast cancer.

**Take-home message**
The clotting protein Tissue Factor may play an important role in breast cancer stem cell activity and may be a potential target in the treatment of breast cancer.

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**O72 INVESTIGATING THE ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS AND INDELS IN PCM1 (PERICENTRIOLAR MATERIAL) GENE AND BREAST CANCER IN THE WEST OF IRELAND**
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**Introduction**
Breast and ovarian cancers share some common genetic susceptibility (eg BRCA1, BRCA2 mutations, KRAS variant). High through-put sequencing of DNA from patients with ovarian cancer for putative disease-causing mutations has identified two variants (Insertion ATTT at 8p21.3-22, and base substitution G>A 9base pairs upstream) in the 3' UTR of the PCM1 gene (Pericentriolar Material-1). The aim of our study was to examine the association of these genetic variants with breast cancer in a population from the west of Ireland.

**Method**
DNA was extracted from whole blood using a salting-out technique. Seven hundred and sixty-eight DNA samples (384 cases, 384 controls) were sequenced for PCM-1 using bi-directional Sanger sequencing.

**Results**
Both variants were identified in our population, and were found to exist independently as well as concomitantly. Fourteen per cent (n=54) of cases and 18% (n=66) of controls were homozygous for insertion ATTT, and the majority of cases (n=242, 63%) and controls (n=227, 60%) heterozygous for this variant (p=0.43, X2). There was no significant difference in the prevalence of the Single Nucleotide Polymorphism between cases and controls (p=0.434, X2). Five different combinations of the two variants were noted to occur in our cohort, but there was no difference in their distribution between cases and controls.

**Conclusion**
Novel variants in the PCM1 gene have been shown to be enriched in ovarian cancer but not in breast cancer. These variants were found to exist independently as well as in combination, highlighting that genetic mutations may co-exist, and therefore may potentially interact.

**Take-home message**
Two novel variants in the PCM1 gene have been identified that exist independently and concomitantly in patients from the west of Ireland.

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**O73 14-3-3 PROTEINS, TBID, AKT1 AND FAKY397 MAY BE PREDICTIVE BIOMARKERS OF RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN OESTROGEN RECEPTOR POSITIVE BREAST CANCER AS REVEALED BY COMBINED ANTIBODY MICROARRAY AND 2D-PAGE/MALDI/TOF/TOF/MS PROTEOMICS**
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**Introduction**
Neoadjuvant chemotherapy is a standard treatment for locally advanced breast cancer however chemoresistance can be a major obstacle in oestrogen receptor (ER) positive cancers. Using comparative proteomic approaches (antibody microarray/AbMA and 2D-PAGE with MALDI-TOF/TOF MS) to investigate a pilot series of breast cancer samples we recently identified 14-3-3 theta/tau, tBID and Bcl-XL as putative biomarkers of response
to neoadjuvant chemotherapy (Hodgkinson et al J Prot 2012, 75:1276-1283 and 75:2745-2752). Here we aimed to analyse further samples using the AbMA approach and to re-analyse the combined data.

Method
Samples from chemoresistant and chemosensitive breast cancers were selected following anthracycline-taxane chemotherapy. Differential protein expression was compared between chemoresistant and chemosensitive samples using the Panorama XPRESS Profiler725 AbMA kit. The combined data from 9 AbMA assays and 3 2D-PAGE/MS experiments was then analysed using Ingenuity Pathway Analysis. A pilot series of archival samples was used for clinical validation of putative predictive biomarkers.

Results
55 differentially expressed proteins (DEPs) were seen in the 4 further AbMA experiments. In the combined dataset (12 experiments from 2 proteomic platforms), 8 DEPs were seen in at least 3 experiments. Clinical validation in a pilot series of archival samples revealed 14-3-3 theta/tau, tBID, AKTphosphoser473 and FAK phosphoY397 to be significantly associated with chemotherapy resistance.

Conclusion
We have identified at least 8 proteins which could play a role in chemoresistance. We propose a potential role for AKT-1, FAKY397, 14-3-3 theta/tau and tBID as predictive biomarkers of neoadjuvant chemotherapy resistance in breast cancer.

Take-home message
14-3-3 theta/tau, AKT1, tBID and FAKY397 proteins may be responsible for anthracycline-taxane breast neoadjuvant chemotherapy resistance.

O74 WIDE LOCAL EXCISION DEFECTS OF THE BREAST – A TREATMENT ALGORITHM BASED ON ONCOLOGICAL RESECTION PATTERNS
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Introduction
Reconstruction of wide local excision (WLE) defects can be performed using various autogeneous and alloplastic techniques. Options include lipofilling, oncoloplastic reductions, TDAP flaps, LICAP flaps, free flaps and silicone implants. We present an algorithm based on the breast quadrant involved, the presence or absence of nipple deviation and the size of the defect which helps in the selection of the most appropriate reconstructive technique.

Method
From 2008 to date we have reconstructed 28 WLE defects. Patients were categorised according to breast quadrant involved, volume deficit and nipple position. Techniques used included lipofilling (n=7), oncoloplastic reductions (n=9), TDAP flaps (n=8), ICAP flaps (n=1), DIEP flaps (n=2) and implants (n=1).

Results
For UOQ (n=7) and LOQ (n=3) defects with nipple deviation TDAP and DIEP flaps were used. The LIQ (n=1) defect was reconstructed with an ICAP flap. UIQ (n=5) defects without nipple deviation were managed with lipofilling. For central defects (n=1) an implant was utilised. In patients with macromastia or ptosis, oncoplastic breast reduction was possible. One TDAP flap became congested requiring partial debridement and later lipofilling.

Conclusion
Our treatment algorithm aids in the selection of the most appropriate reconstructive option for the treatment of this challenging problem.

Take-home message
Various surgical options are available in the reconstruction of WLE defects in the breast, our treatment algorithm aids in the selection of the most appropriate.

O75 IDENTIFYING NOVEL BREAST CANCER SUBTYPES EFFECT ON CLINICAL RESPONSE TO NEOADJUVANT CHEMOTHERAPY
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Introduction
Neoadjuvant chemotherapy (NCT) is used in locally advanced/operable breast cancer. Gene expression analysis has identified several prognostically different breast cancer subtypes. Our study aimed to identify individual tumour factors based on such subtyping to maximize Neoadjuvant chemotherapy response rates.

**Method**
57 women with operable breast cancer receiving Neoadjuvant chemotherapy (January 2010-June 2013) were retrospectively identified. Clinical/radiological response was 50% reduction of the sum of the largest tumour and lymph node dimensions following NCT. Complete pathological response (pCR) was no residual tumour in breast or lymph nodes post surgery Hormone Receptor (HR) and Human Epidermal Growth Factor Receptor 2 (HER2) expression classified cancer subtypes into HR+/HER2−, HR+/HER2+, HR−/HER2+, and HR−/HER2−. A proliferative index marker (Ki-67) subdivided HR+/HER2− tumours, into Luminal A or B.

**Results**
Clinical/radiological response was lowest in HR− HER2−(50%) and highest in HR+ HER2+ (85%) thereby indicating Hormone Receptor (HR+) and HER2 positivity as positive Neoadjuvant Chemotherapy response predictors (p=0.215) Eleven (19%) patients had a complete pathological response (pCR), being highest in the ER− HER2+ subtype (66%) (p=0.001) Tumours with high proliferation index (Ki67) had highest pCR rates in the HR− HER2− subtype (p=0.0047) The clinical/radiological response was not predictive of the pCR in any subtype.

**Conclusion**
In one of the largest Irish series, we identified the HR− HER2+ subtype as the most neoadjuvant chemotherapy sensitive and that a high proliferation index (Ki67) in HR− HER2− subtype is associated with maximal pCR rates. This has exciting therapeutic potential in identifying patients most suitable for neoadjuvant chemotherapy.

**Take-home message**
The study aims at identifying patients most suitable for neoadjuvant chemotherapy, in order to maximize the neoadjuvant chemotherapy response rates.

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**O76 A NEW SURGICAL PROTOCOL TO REDUCE RE-OPERATION RATES IN BREAST CONSERVING SURGERY**
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**Introduction**
The challenge of breast conserving surgery (BCS) with Wide Local Excision (WLE) is to balance oncological control with good long-term, aesthetic results. This study presents the results of an audit in a single breast centre and the introduction of a local protocol to reduce re-excision rates.

**Methods**
Re-excision rates were reviewed over six months for consecutive patients undergoing BCS for invasive disease. A new protocol was then introduced where additional cavity shave excision biopsies were taken at the time of WLE and re-excision rates were reviewed. Histological assessment was performed by a dedicated breast pathologist and re-excision recommended if invasive or in-situ disease was found within 2mm of any new resection margin.

**Results**
In the initial cohort of 81 patients (age range 25.9 – 82.1 years, median 62.5), 27 (33%) had close or involved margins. Following the new protocol there was no significant difference in the mean weight of WLE specimens (34.6g vs 33.5g, p=0.720 on T-test) or the mean diameter of invasive disease in each cohort (16.8mm vs 19.6mm, p = 0.100 on T-test). In the second group of 81 patients (age 34.7 – 83.4 years, median 63.7 years), only 15 (18.5%) (p=0.048, Chi-square = 3.89, dF = 1) required a second operation due to involved margins.

**Conclusions**
The introduction of cavity shaves can significantly reduce re-excision rates following BCS in invasive breast cancer. This does not affect the total volume of tissue resected and we infer that this allowed us to optimise oncological treatments with equivalent breast cosmesis.

**Take-home message**
The introduction of cavity shaves can significantly reduce re-excision rates following BCS in invasive breast cancer.
ALTERED GLYCOSYLATION OF IgA1 IN BREAST CANCER
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Introduction
Most proteins are modified by the attachment of complex sugars in a process known as glycosylation. Alterations in protein glycosylation have been reported in cancer and are associated with metastasis formation. Glycosylation may be studied using carbohydrate binding proteins (lectins) and using this approach glycoproteins (e.g. immunoglobulin A, IgA1) have been identified as aberrantly glycosylated in metastatic cancer. In this study the glycosylation of serum IgA1 from patients with breast cancer was compared with IgA1 from age-matched healthy individuals.

Method
Ethical approval was granted by the North London Research Ethics Committee. Serum IgA1 levels were measured by ELISA and the glycosylation assessed by incorporating lectins (Helix pomatia agglutinin, HPA: N-acetylgalactosamine; Sambucus nigra agglutinin, SNA and Maackia amurensis lectin: MAL-II: sialic acids) into the detection system. Affinity purified IgA1 was used in detailed glycan mapping experiments. Immunohistochemistry was used to assess whether IgA1 is present in breast cancer tissues.

Results
Breast cancer serum IgA1 showed an increase in N-acetylgalactosamine residues (P=0.02). Glycan mapping experiments revealed increasingly sialylated N-linked glycan structures in the cancer serum samples (P<0.0001: non-core fucosylated; P=0.0345: core fucosylated). An increase in the level of asialo-TF and disialo-TF antigens was also observed in the glycan preparations from the cancer samples. Immunohistochemical analyses showed anti-IgA1 antibodies react with breast cancer tissue sections concuring with previous reports that IgA1 is detectable in cancerous tissues.

Conclusion
Altered glycosylation of IgA1 has been observed in breast cancer; this may modulate IgA1 function and may be a useful biomarker for breast cancer prognostication.

Take-home message
Altered glycosylation of IgA1 has been observed in breast cancer; this may modulate IgA1 function and may be a useful biomarker for breast cancer prognostication.