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ABSTRACTS

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Highlights of 2017 (II)—Early Disease

Professor Michael Gnant, MD, FACS

Medical University of Vienna, Vienna, Austria

A number of important **large clinical trials** in the field of early breast cancer (EBC) reported results at large international meetings such as ASCO, ESMO, and SABCS in 2017:

In the field of **extended adjuvant endocrine** therapy, the IBCSG/BIG SOLE trial designed to evaluate the use of intermittent letrozole to prolong sensitivity to endocrine treatment in postmenopausal women with HR + BC showed that extended intermittent letrozole did not improve DFS versus continuous dosing. The ABCSG-16 trial of additional 2 versus additional 5 years of anastrozole after 5 years of endocrine therapy was presented at SABCS.

In **HER2-overexpressing** breast cancer, the adjuvant APHINITY trial demonstrated a significantly reduced risk of recurrence for the addition of pertuzumab versus placebo to trastuzumab and chemotherapy in patients with HER2+ EBC (HR: 0.81, $p = 0.045$), with patients with node-positive and or HR− disease benefitting the most. The adjuvant ExteNET trial of "secondary" neratinib after the completion of trastuzumab in its 5-year update showed improved DFS compared to placebo (HR 0.73, $p = 0.008$), but the benefit was almost exclusively driven by the HR+ cohort of patients (HR = 0.60, $p = 0.002$). A comparison of 9 weeks of adjuvant trastuzumab versus 1 year (SOLD trial) was presented at SABCS.

With respect to **neoadjuvant endocrine therapy**, the phase II LORELEI trial demonstrated a significant increase in overall response rates measured by centrally assessed MRI for the addition of the PI3 K selective inhibitor taselisib to letrozole in ER-positive/HER2-negative breast cancer, with some indication that this treatment effect was more pronounced in the *PIK3CA*-mutated population. The POETIC trial assessing the comparative clinical utility of Ki67 at baseline and after 2 weeks of pre-operative AI in predicting long-term outcome was presented at SABCS.

In the field of **chemotherapy**, the PlanB trial reported the non-inferiority of the TC chemotherapy scheme compared to EC-T. In a phase-III neoadjuvant trial for triple-negative breast cancer, the addition of carboplatin to AC led to improved pCR rates, but the addition of the PARPi veliparib did not further increase pCR rates. In the field of introducing immunotherapeutic approaches to BC, the I-SPY 2 program demonstrated that the addition of pembrolizumab can increase pCR rates both in triple-negative and HR+/HER2− tumors.

Should radiotherapy be routinely recommended after wide local excision of DCIS?

David Dodwell

Institute of Oncology, St James' Hospital, Leeds, UK

The use of whole breast radiotherapy (WBRT) after wide excision for DCIS is commonplace but variable practice exists across the world and there is substantial geographical variation within the UK. This variability is fuelled by the recognition that the great majority of DCIS is screen-detected and patients with this condition have an excellent prognosis. Other cause mortality is, in most circumstances, much greater than breast cancer-related mortality and there is therefore a serious risk of overtreatment. Additionally, although WBRT reduces the risk of invasive or in situ within-breast recurrence, the five randomized controlled trials (RCTs) of WBRT versus no WBRT after wide excision and the EBCTCG meta-analysis of four of these RCTs have shown equivalent overall-survival and breast cancer-related mortality. Long-term morbidity from WBRT is very unusual but does occur and the small risks of cardiac toxicity and carcinogenesis need to be considered.

The publication of results derived from selected cohorts of patients with very low recurrence rates without WBRT has encouraged many to focus attention on improvements in surgical techniques and margin assessment as a means of avoiding a need for WBRT. Unfortunately the very low recurrence rates reported in selected series have not been reflected in larger population based cohorts.

Efforts to improve the selection of radiotherapy by the identification of patients at higher risk of recurrence have met with mixed result. These efforts have included the development of prognostic scores such as the Van Nuys Index, nomograms using other demographic and tumour-related factors and molecular classifiers such as the Oncotype DX test for DCIS.

The long natural history of this disease and the associated low event rates have meant that randomized prospective studies are relatively lacking and alternatives usually involving observational evidence have been more prominent. This is understandable and such information is very useful for the study of long-term outcomes but is less helpful to study the impact of surgery and adjuvant therapy because of the many possible confounders that exist.

At this point in time, definitive recommendations as to which patients require WBRT, based on high level evidence, cannot easily be made and we continue to strive to strike a balance between under and over treatment on an individual basis. The ongoing collection of outcome information in routine practice and prospective trials linked to efforts to derive improved molecular indicators of recurrence risk and radiotherapy benefit are a priority.

The use of patient-derived tissues for advanced preclinical models of breast cancer

Robert Clarke, PhD

Breast Cancer Now Research Unit, The University of Manchester, UK

Recently, there has been an interest in improving breast cancer models through the development of patient-derived organoid (PDO) 3D cultures and xenograft (PDX) tumours. Where these PDO/PDX have been characterised, they mostly retain the principal histologic and genetic characteristics of their donor tumour, which remain stable across several passages. PDO 3D cultures and PDX tumour models are now available that represent the different molecular subtypes of breast cancer. PDX have been shown to undergo metastasis to lymph nodes, liver, lung and bone, making them useful for biological and preclinical modelling of patient disease. Both PDO and PDX will be vital emerging toolsets for preclinical drug evaluation, biomarker identification, biological studies and personalised medicine strategies.

We prospectively collected more than 300 primary breast cancer samples and assessed 3D mammosphere colony-forming activity in vitro in both primary (formation) and secondary (self-renewal) culture, and tumour-initiating activity in vivo.

Metastatic samples formed more mammosphere colonies than EBC and had higher primary mammosphere colony formation. Tumour initiation in vivo was significantly higher in metastatic versus EBC samples (63 vs. 38%; $p < 0.05$). We established 20 stable PDX models at passage 2 or greater from subcutaneous implantation of breast cancer samples. Metastases were detected in lungs of mice from 14/34 PDX models and this correlated significantly with mammosphere colony formation in vitro. PDX models are now being utilised to study the early stages of metastasis.

In summary, colony formation and tumour initiation are increased in metastatic compared to early breast cancer samples, and predict metastasis in vivo. Thus, breast stem cell activity may predict for poor outcome tumours, and therapy targeting this will reduce secondary breast cancer.

3D in vitro modelling using material from Breast Cancer Now Tissue Bank

Dr Richard Grose

Barts Cancer Institute, Queen Mary University of London, UK

3D modelling fulfils a critical role in research, allowing for complex cell behaviour and interactions to be studied in physiomimetic conditions. With tissue banks becoming established for a number of cancers, researchers now have access to primary patient cells, providing the perfect building blocks to recreate and interrogate intricate cellular systems in the laboratory. Understanding the relationship between myoepithelial and luminal cells in the development of breast cancer is critical for the development of new therapies and prognostic markers. This requires the generation of new models that allows for the manipulation of these two cell types in a physiological setting.

Using access to the Breast Cancer Now Tissue Bank, we isolated pure populations of myoepithelial and luminal cells from human reduction mammoplasty specimens and placed them into 2D culture. These cells were infected with lentiviral particles encoding either fluorescent proteins, to facilitate cell tracking, or an inducible human epidermal growth factor receptor 2 (HER2) expression construct. Myoepithelial and luminal cells were then recombined in collagen gels, and the resulting cellular structures were analysed by confocal microscopy.

Myoepithelial and luminal cells isolated from reduction mammoplasty specimens can be grown separately in 2D culture and retain their differentiated state. When recombined in collagen gels, these cells reform into physiologically reflective bilayer structures. Inducible expression of HER2 in the luminal compartment, once the bilayer has formed, leads to robust luminal filling, recapitulating ductal carcinoma in situ, and can be blocked with anti-HER2 therapies.

This model allows for the interaction between myoepithelial and luminal cells to be investigated in an in vitro environment and paves the way to study early events in breast cancer development with the potential to act as a powerful drug discovery platform.

Breast cancer research: past and future

Marc Lippman MD MACP FRCP

University of Miami Miller School of Medicine, USA

We all can and should take collective pride in the many significant achievements in the detection prevention and treatment of breast cancer that have taken place in the past decades. The outlook for patients is far better than it once was. This presentation will focus on some of the things that we do not know—the answers to which might further improve the outlook for patients. Here are some of the questions that continue to trouble me. Why is a treatment [tamoxifen, for example] capable of preventing a significant number of breast cancers and curing others in the adjuvant setting, unable to cure essentially anyone with even the most minimal metastatic disease? Put another way, why is a woman's prognosis significantly better with bulky locally advanced breast cancer than with a single metastatic focus of small volume? Why do patients with multiple scattered breast cancer cells in lymph nodes have a prognosis indistinguishable from women with negative nodes? And why do women with 3 versus 2 versus 1 axillary lymph node metastases all have differing prognoses? Are positive lymph nodes the source of metastases or an epiphenomenon? How can post-mastectomy radiation improve survival in some patients while not doing so when combined with lumpectomy? Why do women with readily detected circulating breast cancer cells and positive bone marrows not develop metastatic disease even when followed for many years? Put another way, why, unlike lung, colorectal or many other cancers are 5 years without recurrence unreliable as a predictor for cure? In fact, do we ever really cure breast cancer—or at least ER-positive breast cancer? Why do changes in woman's lifestyle such as weight gain, diabetes, stress, depression and failure to exercise all impact recurrence of breast cancer? As stated by Winston Churchill: "Men occasionally *stumble* over the *truth*, but most of them pick themselves up and hurry off as if nothing ever happened".

Neoadjuvant Therapy as a Surgical Tool

Professor JM Dixon

Western General Hospital, Edinburgh, UK

One advantage of neoadjuvant therapy is the ability to assess response of the cancer to chemo or hormone therapy. The neoadjuvant setting provides an opportunity to investigate changes in the cancer during treatment and to explore mechanisms of sensitivity and resistance.

Although classically neoadjuvant means a prolonged course of treatment before surgery, short periods of pre-operative treatment—so-called window studies can also provide an opportunity to personalise treatment based on changes/response in the individual and their cancer. It is the changes seen in response to treatment that may provide a more meaningful prediction of long-term outcome than a single assessment of the cancer at diagnosis.

Data show that response to chemotherapy is a powerful predictor of outcome. This will be discussed in detail by Dr Fraser Symmans. Ongoing studies in patients treated with neoadjuvant chemotherapy are attempting to identify predictors of response but are complicated by the very many different phenotypes of breast cancer and variable response rates.

Neoadjuvant endocrine studies of essentially Luminal A breast cancers have proved more successful and have shown that short-term changes in the cancer are powerful predictors of long-term outcome. In the neoadjuvant setting, it has also been possible to investigate primary and acquired endocrine resistance mechanisms. These studies have given much greater insight into mechanisms of resistance than cell line studies. The neoadjuvant setting has also allowed us to understand dormancy and the pathways involved in the later awakening of dormant cells to cause relapse.

Finally, the neoadjuvant setting provides an opportunity to determine whether all patients treated by systemic therapy require surgery as well as radiotherapy. Studies of core biopsies in the post chemotherapy setting have shown that as yet, surgery is still needed to define response.

Treatment of the axilla post neoadjuvant chemotherapy

Judy C. Boughey, MB, BChir, FACS, Professor of Surgery

Mayo Clinic, Rochester, MN, USA

Neoadjuvant chemotherapy (NAC) is known to decrease the extent of disease in the breast and increase rates of breast conservation. In addition, NAC also can reduce the likelihood of nodal positivity and hence decrease need for axillary node dissection and its associated morbidities.

Three prospective clinical trials have assessed the false-negative rate (FNR) of SLN after NAC for patients with clinically node-positive disease at presentation. The American College of Surgeons Oncology Group (ACOSOG) Z1071 study reported a false-negative rate (FNR) of SLN surgery of 12.6% in patients with cN1 disease with two or more SLNs resected. This was lower at 10.6% when dual tracer technique was utilized. Additional analysis showed that when a clip was placed in the positive node at diagnosis and the clipped node was resected as one of the SLNs, the FNR was 6.8%.

The Canadian study (SN FNAC—sentinel node following neoadjuvant chemotherapy) reported a FNR of 13.3% when defining SLN with isolated tumor cells (ITC) as negative and 8.4% when including ITC in the definition of a positive SLN. The SENTINA study from Europe reported an overall FNR of 14.2%, however when excluding patients with only a single SLN removed the FNR was 9.8%.

Further work with preoperative localization of the clipped node with a seed and resection of the localized clipped node along with the sentinel nodes (termed targeted axillary dissection) has been shown to have a FNR of 2.4%. Surgeons are incorporating SLN after NAC into their clinical practice for patients with a good response to NAC.

For patients with biopsy-proven node-positive breast cancer, SLN surgery after NAC allows assessment of residual nodal disease and can enable patients who have their axillary disease eradicated by NAC to avoid ALND.

Imaging endpoints for neoadjuvant therapy

Dr Sarah J Vinnicombe

Ninewells Hospital and Medical School, University of Dundee, UK

As increasing numbers of patients with both locally advanced and early-stage breast cancer are treated with neoadjuvant therapy, assessment of response to treatment has become crucial in the application of personalised precision medicine. Imaging has a pivotal role in response assessment, but it is important to recognise that choice of the most appropriate imaging biomarker or endpoint depends on the precise clinical question as well as the nature of the neoadjuvant therapy. Ideally, baseline imaging should accurately predict the likelihood of response to the planned neoadjuvant treatment, since avoidance of a potentially toxic therapy that is unlikely to be effective would benefit the patient and the healthcare system. To date, however, the predictive power of baseline imaging is generally insufficient to direct treatment for the individual patient, even when multiparametric functional techniques such as breast magnetic resonance imaging (MRI) are used.

At the end of treatment, there are two main considerations. Firstly, the ability to confidently identify patients who have attained a pathological complete response (pCR) might enable the avoidance of surgery altogether in certain subgroups, necessitating a very high negative predictive value for the imaging test in question. On the other hand, accurate depiction of the amount of residual disease is essential for accurate surgical planning, necessitating a high positive predictive value. The shortfalls of morphological and functional imaging in the literature largely reflect failure to recognise the critical impact of tumour immunophenotype on both likelihood of pathological response and the diagnostic accuracy of imaging. Consequently, there is marked national and international variation in the amount and nature of imaging used during neoadjuvant therapy. In the future, it is likely that radiogenomics will provide novel insights and guidance, but in the meantime, accurate image-guided sampling of the tumour bed and axilla is essential to direct response-adapted treatment.

Applying digital pathology to translational breast cancer research

Professor Valerie Speirs

University of Leeds, UK

Evaluation of tissue images is traditionally performed by inspecting stained tissues mounted on glass slides using a light microscope. However this can be subjective, labour intensive and is often subject to considerable intra- and inter-observer variability, particularly for immunohistochemistry (IHC). In recent years, great strides have been made following the development of automated image analysis systems and a number of these are now on the market. Computer-based analysis of digital histopathology images using virtual slides is attractive as it can, potentially, improve the accuracy and efficiency of interpreting IHC staining by eliminating subjectivity and intra-observer variability. Virtual slides can be used to turn 2D images into 3D tissues, providing greater insight into tissue architecture. Here, I will outline how some of these methods are being used in translational breast cancer research.

Imaging-based analysis of ErbB/HER signalling dimer and immunoregulatory molecules—from preclinical science to biomarker discovery

Professor Tony Ng

King's College London, UK

Drug resistance is currently an inevitable consequence of drug therapy for solid cancers and greater understanding of underlying resistance mechanisms using novel tools that can be translated into the clinical setting to guide treatment selection is critical to improve clinical cancer outcomes. I will describe the impact of an important compensatory/drug resistance mechanism we have termed therapy-induced ErbB/HER/Met receptor tyrosine kinase (RTK) rewiring; whereby tumours evolve, under treatment pressure, a conformational alteration in ErbB/HER receptor(s) favouring the formation of increased dimerization. The receptor dimerization was quantified in FFPE patient samples using an in-house fluorescence lifetime imaging (FLIM) histology platform. The clinically important link to be established is how this non-canonical receptor rewiring process, induced by therapies, can in turn influence the tumour microenvironment. For elucidating this link we are focusing on unfolded protein response-based mechanisms that can modulate the adaptive and innate immune effectors in the tumour microenvironment and can be probed in exosomes. I will also describe our imaging-based observation that there is an ALIX- and EGFR-dependent receptor sorting mechanism which can package immunoregulatory molecules such as PD-L1 onto intraluminal vesicles (ILV) of multivesicular bodies (precursors of exosomes) and thereby influence the

immunosuppressive function of these checkpoint molecules. These preclinical observations will be supported by correlating the images of PD-L1 with the ALIX mRNA expression in breast cancer patient tissues.

Deciphering spatial heterogeneity of tumor immune response

Yinyin Yuan

Institute of Cancer Research, London, UK

Despite increasing evidence supporting the clinical utility of immune infiltration in the estrogen receptor-negative (ER−) subtype, the prognostic value of immune infiltration for ER+ disease was not well defined.

We performed a study to quantify lymphocyte abundance and spatial heterogeneity using fully automated hematoxylin and eosin-stain image analysis algorithm and spatial statistics for 1178 post-menopausal patients with ER+ breast cancer treated 5 years with tamoxifen or anastrozole. Prognostic significance of immune scores was compared with Oncotype DX 21-gene recurrence score (RS), PAM50 risk of recurrence (ROR) score, IHC4, and clinical treatment score (CTS) available for 963 patients.

We report that scores of immune cell abundance were not associated with recurrence-free survival. In contrast, high immune spatial scores indicating increased cell spatial clustering were associated with poor 10-year, early (0–5 year), and late (5–10 year) recurrence-free survival. The prognostic value of spatial scores for late recurrence was similar to that of IHC4 and RS in both populations, but was not as strong as other tests in comparison for recurrence across 10 years.

Of note, in contrast to our observation in ER+ tumors, high Immune-Cancer Hotspot score, indicating increased spatial clustering in immune and cancer cells, correlated with favorable prognosis in ER-breast cancer ($n = 246$). Our findings support different mechanisms implied in treatment resistance in breast cancer subtypes, and call for the development of novel cancer therapeutics targeting the pathways that bypass these mechanisms.

Our results provide a missing link between tumor immunity and disease outcome in ER+ disease by examining tumor spatial architecture. The association between immune spatial scores and late recurrence suggests a lasting memory of pro-tumor immunity that may impact on disease progression and evolution of endocrine treatment resistance, which may be exploited for therapeutic advances.

The Benefits of Shared Decision Making

Prof Diana Harcourt, Dr Nicole Paraskeva & Prof Alex Clarke

Director, Centre for Appearance Research, University of the West of England (UWE, Bristol), UK

Decisions about treatment after diagnosis of breast cancer can be complex but they often need to be made in a relatively short period of time, amidst the emotional turmoil and upheaval associated with cancer. It is perhaps not surprising, therefore, that patients often describe decision making as being difficult and many report dissatisfaction with outcomes and regret about the choices they have made.

Shared decision making has been heralded as a means of improving patient-reported outcomes and satisfaction with cancer care, particularly concerning decisions that are 'preference sensitive' (i.e. those where the 'right choice' depends on individuals' personal preferences rather than generic treatment factors), such as options concerning breast reconstruction. This presentation will consider the challenge of implementing shared decision making, evidence that it can be beneficial for patients, health professionals and policy makers who are interested in 'getting it right first time', and suggest that it should be central to today's breast reconstruction services.

What can help to make a good decision in a difficult situation like breast cancer?

Prof Alex Clarke, Dr Nicole Paraskeva & Prof Diana Harcourt

Visiting Professor, Centre for Appearance Research UWE

How can psychological theory be used to inform and underpin decision making? Quality information is essential, particularly for complex, preference sensitive decisions, and this includes information we elicit from the patient as well as what we provide to them. But ultimately, it is not just the information itself that underpins a shared decision-making approach, but rather the process of how that information is being used.

Understanding some of the heuristics and biases that impact on how patients process information and how our own behaviour as health professionals can bias the choices they make is important. This session draws on clinical experience and the process of developing a tool to facilitate shared decision making to discuss practical strategies to helping people make decisions that are both well informed and consistent with their core values and beliefs.

Tools available to support shared decision making about breast reconstruction

Dr Nicole Paraskeva, Professor Diana Harcourt, Prof Alex Clarke

Centre for Appearance Research, University of the West of England, Bristol

Decision making about breast reconstruction (BR) following a diagnosis of breast cancer, Ductal Carcinoma in Situ (DCIS) or to reduce future breast cancer risk is difficult and complex. Indeed, choices regarding whether to undergo reconstruction, the type (e.g. implant based, autologous) and timing (immediate, delayed,) of surgery are considerable, and the best option for each woman will depend on her own individual preferences, goals and needs.

Given these complexities and challenges, a variety of interventions have been developed to support shared decision making about BR. This talk will review the available interventions, discuss their content, format and effectiveness in improving patient-reported outcomes, including decisional conflict, decisional regret, treatment choice, satisfaction, anxiety and depression. The methodological quality of research will also be assessed. Finally, this talk will identify directions for future research in this area in order to advance the development of effective interventions to support decision making about breast reconstruction.

Triple-Negative Breast Cancer—the biology of new targets

Andrew Tutt MB ChB PhD, Director Breast Cancer Now Research Centre

Institute of Cancer Research and King's College London

The term Triple-Negative Breast Cancer (TNBC) implies a single biological entity, which is a not the case. While clinically convenient, this term hampers rational biologically targeted therapy and proof of concept clinical trial development. TNBC covers at least four biological entities with additional overlying variability in tumour immune stroma. Basal-like breast cancers are the most common TNBC subtypes and show highest levels of genomic instability dominated by chromosomal copy number aberrations (CNAs), suggesting underlying aberrant DNA damage responses and commonly show MYC upregulation both of which cause high levels of DNA replication stress. A significant sub-group have DNA homologous recombination repair defects, often driven by *BRCA1* mutation or mRNA silencing but also underpinned by other recently identified drivers such as the meiotic gene HORMAD1. CNAs often lead to loss of expression of PTEN or INPP4 negative regulators of the AKT/PI3-kinase pathway, which can also be activated by amplifications or mutations in AKT isoforms or PI3-kinase itself, as well as amplifications of MYC and MCL1 BCL2 family cell death regulator MCL1 and their "druggable" regulatory kinase PIM1. Non-basal-like forms include a luminal-like androgen receptor (LAR)-regulated group and a mesenchymal gene expression group with the lowest levels of response to neoadjuvant chemotherapy. Expression of immune response genes occurs in a significant proportion of most TNBC subtypes that can be differentiated into those with more anti-tumour activated immune gene expression signatures and immune signatures with less evidence of activation. The mesenchymal subtype very rarely shows evidence of immune-modulatory activity. As the immune-biology and immunotherapy of TNBC will be covered by others, I will focus on the biological rational and, where possible, early-phase trial data relating to the targeting of DNA replication stress and associated aberrant DNA repair, MYC upregulation, AKT and PI3-Kinase pathway abnormalities and androgen receptor axis.

Precision medicine for Triple-Negative breast cancer patients using a systems biology approach

Charles M. Perou, PhD

The May Goldman Shaw Professor of Molecular Oncology

Department of Genetics, and Pathology & Laboratory Medicine, Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Triple-Negative Breast Cancers are amongst the most clinically challenging because of their poor prognosis and paucity of approved treatment options. TNBC is composed of multiple disease subtypes, and with multiple classification systems having been developed. These methods are largely in agreement and identify four basic groups including Basal-like, Claudin-low/Mesenchymal, Immune-infiltrated, and Luminal-type (LAR). Each of these distinct patient subsets may require distinct therapeutic strategies, some of which will be discussed.

Within TNBC, the Basal-like disease type predominates (70–80%), but again, all the intrinsic subtypes are also present. Interestingly, luminal/TNBC or HER2−-enriched/TNBC shows very similar gene expression patterns to Luminal/ER+ or HER2−-enriched/HER2+, except for expression of ERBB2 (and the genes in the 17q amplicon). Importantly, the distinction between Basal-like versus non-Basal-like within TNBC seems to be important for predicting (1) survival following (neo)adjuvant multi-agent chemotherapy, (2) bevacizumab benefit in the neoadjuvant setting (CALGB40603), and (3) docetaxel versus carboplatin benefit in the first-line metastatic setting (TNT Trial). In addition, genomic predictors of chemotherapy benefit for TNBC typically identify tumor and immune cell features as positive predictors of response, thus highlighting the importance of the microenvironment in response and survival.

TNBC is also more common in younger women, and African American women, which contributes to racial disparities in mortality due to breast cancer. Recent TCGA analyses suggest that there are few molecular differences in the somatic tumor landscape according to race, which suggests that access to healthcare, and/or adherence to therapy may be the major contributors to differences in outcomes according to race. Overall, these data suggest that intrinsic molecular profiling, plus measures of the microenvironment, can provide clinically relevant information beyond current pathology-based classifications, which can assist in making more precise therapy recommendations for TNBC patients.

Immunologic advances

Prof Giuseppe Curigliano MD PhD

Istituto Europeo di Oncologia, Department of Hematology and Oncology, University of Milano

The increasing understanding of the dynamic interactions of the immune system and tumors has inspired researchers to devise a variety of approaches to harness the power of the immune system for the treatment of cancer. Unlike classical therapies, which can be immunostimulating as a bystander effect, immunotherapies are specifically designed to redirect the immune system to destroy tumors. There are at least three major immunotherapeutic strategies in which this can be achieved: passive transfer of anti-tumor immunity, active induction of anti-tumor immune responses using therapeutic vaccination, and immunomodulatory agents which act on the endogenous, naturally acquired anti-tumor immunity. Considerable evidence supports an impact of the host immune system on breast tumor development and responsiveness to therapy. Especially for triple-negative and HER2+ breast cancer, tumor-infiltrating lymphocytes and upregulation of immune-related gene signatures have been associated with a favorable prognosis. Increasing insight into tumor immunogenicity and associated gene expression suggests that the functional molecular orientation of the tumor immune landscape is a key determinant for the effect on patient prognosis. Recent breakthroughs have defined links between breast tumor genetic attributes and intratumoral immune phenotypes, suggesting intrinsic tumor characteristics might shape the tumor immune microenvironment. In this presentation, we give an overview of immune gene signatures in both adjuvant and neoadjuvant treatment settings and their prognostic performances across different breast cancer subtypes. Consistent themes generally reflected in these signatures will be presented and the importance of the development of a consensus classification system is underlined. The role of the functional molecular orientation of the immune infiltrate on patient prognosis and its association with tumor intrinsic characteristics will be discussed. Finally, recent insights into associations between breast cancer genetic attributes and tumor immune phenotypes that reveal important potential implications for the design for immunotherapies will be provided.

Breast cancer prevention for the population at large

Prof Jack Cuzick

Queen Mary University of London, Wolfson Institute of Preventive Medicine

Breast cancer is by far the commonest cancer in women and it is an ideal candidate to develop strategies for cancer prevention. For the general population, weight control and maintaining a good level of physical activity are important, as is routine breast screening with mammography. However not all women are at the same level of risk and an initial risk assessment would be useful to guide risk-adapted screening intervals, as well as identify a small subgroup for which preventive therapy is appropriate.

Two drugs, tamoxifen and raloxifene, are licensed for preventive therapy in the United States and recommended by NICE in the UK. Newer approaches have looked at the role of aromatase inhibitors. These show greater efficacy than tamoxifen, but they are only appropriate for postmenopausal women, whereas tamoxifen can also be used premenopausily. NICE has recently recommended anastrozole for high-risk postmenopausal women.

As both of these classes of drugs (SERMs and AIs) have common side effects and some rare more serious ones, it is important to focus their use among women most likely to benefit. An attractive approach is to integrate risk assessment into the first screening appointment and use this to determine subsequent screening intervals and the need for preventive therapy.

Models have been developed to aid this decision and the Tyrer—Cuzick model appears to be one of the best at the moment. It is now clear that mammographic breast density is an important predictor and recent results indicate that a risk score combining the 88 of the currently identified risk single nucleotide polymorphisms (SNPs) adds to predictive accuracy. Initial data indicate that both density and SNPs (which can be measured in a saliva sample) are sufficiently independent of other factors to make substantial improvements to overall risk prediction.

Stratification of screening

Professor Fiona J Gilbert

University of Cambridge

The UK NHS breast screening programme currently offers three yearly mammography to women between the ages of 50 and 70 years. All other countries offer more frequent mammography or digital breast tomosynthesis (DBT) and some offer supplemental screening with ultrasound to women with increased breast density. It is known that high breast density increases the likelihood of developing breast cancer by two fold in the over 50-year age group and that increased breast density increases the chance of a cancer being missed or not being detected until the tumour is of a larger size. Trials of Automated whole Breast Ultrasound, Contrast-Enhanced Spectral Mammography and Abbreviated Magnetic Resonance Imaging have been reported or are ongoing which will give an indication of whether or not a more stratified approach should be considered. The ability to select women at increased risk means the additional cost of imaging is offset by increased cancer detection. These various imaging modalities will be reviewed. However, it is essential that trials report the type of cancer being found by the additional imaging as the risk of increased over diagnosis needs to be avoided. There is little value in finding additional cancers or cancers at an earlier stage if there is no alteration in survival. The evidence for changing practice in the UK will be examined.

Risk prediction for risk reduction

Professor D. Gareth Evans

University of Manchester

The concept that there is a population 'risk' of breast cancer such as the widely toted 1 in 8 lifetime risk is hugely misleading. In reality, a tiny proportion of the female population actually have a lifetime risk of breast cancer even between 12 and 13%. Standard risk factors including family history of breast and related cancer (especially ovarian) and hormonal and reproductive factors (age at menarche/menopause, first pregnancy, etc.) are used in a number of risk algorithms such as Tyrer-Cuzick (IBIS), Gail IBCS, and BOADICEA to estimate an individual woman's risk. However, addition of information from mammographic breast density and common genetic variants (Single Nucleotide Polymorphisms-SNPs) can add considerably to the accuracy of risk prediction. In our Predicting the Risk of Cancer at Screening study (PROCAS-women aged 46–73), we have shown that using all three factors together accurately divides women into a number of risk categories in a 10-year period. One-third fall into a low ($< 2\%$) risk and a similar proportion into average risk (2–3.5%). The remainder fall into above average risks with 12% in the moderately increased group (5–7.9%) and 6% into the NICE-defined high-risk category (8%+). Using NICE family history algorithms only 3% fall into the moderate- and high-risk categories. NICE recommends offering chemoprevention with tamoxifen (pre-menopausal) or anastrozole (postmenopausal) in women at high risk and considering this in women at moderate risk. Using the triple approach, those eligible for the offer of chemoprevention that can halve breast cancer risk rises from 0.7 to 6% and almost ten-fold risk. These 6% also develop 22% of the post prevalent stage 2+ cancers which means that 11% of stage 2+ cancers could be prevented by treating 6% of the population with a drug that costs the NHS £12 per annum.

Findings from the Generations Study

Professor Anthony Swerdlow, Dr Michael Jones, Dr Minouk Schoemaker

The Institute of Cancer Research

Breast cancer is a disease whose causation involves events and exposures occurring over many decades of life, and involves factors, notably hormonal, that can only be well assessed from contemporaneously taken samples.

The Generations Study was set up in the UK as a long-term cohort study, which since 2003 has recruited $> 110{,}000$ women from the general population of the UK aged 16 years and older. It is planned to follow the cohort for > 40 years. Follow-up questionnaires are sent every 2½ to 3½ years and completeness of follow-up is very high. The design was focussed specifically to maximise the ability to investigate the causation of breast cancer. The talk will describe the study and some of its outputs to date.

Novel clinical trial endpoints: why do we need better trial endpoints?

Professor Judith Bliss, Director ICR-CTSU & Professor of Clinical Trials

The Institute of Cancer Research

Traditionally, clinical trials have focused on long-term disease outcomes such as disease-free survival in early breast cancer and

progression-free survival and overall survival for patients with metastatic disease. Such trials can provide definitive results leading to changes in routine clinical practice and to the licensing of new therapeutic agents; however, it is well recognized that the time taken to conduct such studies and to observe the long-term disease outcome events precludes speedy innovation in therapeutic development. The era of molecular medicine enables the targeting of novel agents to those deemed most likely to benefit and thus opportunities for speedier read-out. Any increase in likely therapeutic effect size which could reduce required sample sizes may however be offset by the stratification of patients into smaller eligible cohorts which provide challenges for recruitment—a family of rare diseases. Developments in biomarker technologies have created the opportunity to monitor treatment activity during and subsequent to administration of novel therapies and thus may provide the opportunity for speeding up the read-out for novel therapeutic evaluation—thus enabling the acceleration of therapies with the highest promise and the potential to curtail development of agents or strategies which appear futile. These developments beg the question "Are all biomarker endpoints equally valid and reliable?" and "How do we know whether a biomarker change is likely to lead to a change in clinical outcomes?" This session plans an interactive discussion as to the minimum "requirements" of novel endpoints (biological or imaging) that we should expect if we are to be able to make reliable inference from a reported trial. Discussion amongst the different disciplines attending UKIBCS offers the opportunity to both enlighten delegates and debate such considerations.

Novel trial endpoints: opportunities and challenges for new surrogate endpoints

Marc Buyse, Chief Scientific Officer

IDDI (International Drug Development Institute)

A surrogate endpoint is intended to replace a clinical endpoint for the evaluation of new treatments when it can be measured earlier than a clinical endpoint. A good surrogate endpoint should predict clinical benefit, harm, or lack of these. The most commonly used statistical approach to evaluate surrogacy is based on two levels of association: the association between the surrogate and the true endpoint, also known as "individual-level" surrogacy, and the association between the treatment effect on the surrogate and the treatment effect on the true endpoint, also known as "trial-level" surrogacy. These concepts will be illustrated using randomized clinical trials in breast cancer, first in the neo-adjuvant setting, where interest has focused on pathological complete response as a potential surrogate for disease-free or overall-survival, and second in the metastatic setting, where progression-free survival has been evaluated as a potential surrogate for overall-survival in patients receiving anthracyclines or taxanes, as well as in patients with HER2-positive metastatic breast tumors receiving HER2-targeted agents. These examples will illustrate the challenges in evaluating the potential surrogacy of biomarker-based surrogate endpoints, using biological or imaging data. As an illustration, circulating tumor cells have been assessed as potential surrogates for survival in metastatic prostate cancer. While there is much potential for such biomarkers in the future, there is a corresponding need for analytical, clinical, and statistical validation criteria to be prospectively defined.

Genomic heterogeneity of breast cancer

Dr Lucy R Yates

The Royal Marsden Hospital, London

The emergence of commercially available next-generation sequencing (NGS) technologies over the last 10 years has resulted in a paradigm shift in our understanding of the genomic heterogeneity of breast cancer. This presentation will focus on the nature, extent and clinical relevance of genomic heterogeneity between individuals' cancers (inter-tumour heterogeneity) and within individuals' cancers (intra-tumour heterogeneity).

Large-scale international collaborative sequencing efforts have now profiled thousands of breast cancer genomes or exomes (all coding genes). Bioinformatic tools for analysing this so-called 'big data' have identified at least 100 likely cancer genes (genes under positive selection) that drive breast cancer development and most of these are present at a low level across the breast cancer population (present in less than 2–5% breast cancers). The average breast cancer contains driver mutations in just four cancer genes but given the wide range of possible cancer gene combinations, a very broad range of genotypes exist. This poses a major challenge to genomic biomarker-driven clinical trial design and strategies proposed to address this will be presented.

The main message from studies of intra-tumour heterogeneity is that breast cancers are relentlessly evolving entities. A wide range of methods have been incorporated with NGS to study heterogeneity, these include the following: Deep sequencing of bulk-tissue samples; Multi-sampling approaches across space, time or treatment; Single cell approaches and liquid biopsies. These studies confirm that subclones within the tumour can pose problems for representative sampling and can give rise to the aggressive features of a cancer—treatment resistance, metastasis and relapse.

Experimental modelling of heterogeneity

Dr Rachael Natrajan

The Institute of Cancer Research

Solid tumours display significant histological, genetic and micro-environmental intra-tumour heterogeneity that can change substantially over the course of their evolutionary trajectory. In particular, changes in the micro-environmental complexity within breast cancer such as hypoxic and nutrient-deplete environments are associated with aggressive disease and a poor patient outcome.

Here we sought to identify novel driver alterations in aggressive disease by employing a functional genomics screen in a 3-dimensional model of breast cancer progression that more accurately recapitulates in vivo micro-environmental heterogeneity. Screening of the top 200 recurrently mutated genes in breast cancer in cancer cell line spheroids identified several genes whose silencing impacted growth. A second targeted validation screen showed that silencing of a cohort of these genes, including the histone acetyltransferase CREBBP, promoted growth in 3D but had limited effect under traditional 2D culture conditions in a both the MCF10 progression series and a larger panel of triple-negative cell line models.

Investigation of TCGA and METABRIC datasets showed that CREBBP was more frequently mutated in triple-negative breast cancers (TNBCs) and at least a third of TNBCs also displayed gene haploinsufficiency or complete loss of CREBBP. Interrogation of expression and proteomic datasets showed that loss of CREBBP resulted in the up-

regulation of the pro-proliferative transcription factor FOXM1. Significantly, this conserved FOXM1-driven transcriptional programme was also seen in multiple solid tumours with CREBBP alterations including lung, oesophageal, bladder and endometrial cancers. This was recapitulated in several CREBBP deficient cells where we identified that FOXM1 is driving altered metabolism, allowing cancer cells to grow under nutrient-stress conditions.

New selective oestrogen receptor downregulators

Dr Lesley-Ann Martin

Institute of Cancer Research

Targeting ER signalling is the main therapeutic option for patients with oestrogen receptor-positive (ER+) breast cancer (BC). Unfortunately, over 40% of patients relapse with endocrine-resistant disease emphasising the need for improved therapeutic strategies. The prevalence of *ESR1* mutations in relapsed tumours highlights the sustained reliance of advanced tumours on ER signalling, providing a strong rationale for continued targeting of ER. Unlike other endocrine therapies such as aromatase inhibitors (AIs) and tamoxifen, selective ER downregulators (SERDs) are competitive ER antagonists that induce a conformational change in ER resulting in ubiquitination and degradation via the proteasomal pathway. The SERD fulvestrant has shown clinical utility in advanced BC but is limited by its poor pharmaceutical properties, requiring administration via intramuscular injection limiting the dose, exposure, and ER engagement, highlighting the need for SERDs with enhanced bioavailability and pharmacokinetic properties. Here I will discuss new orally bioavailable SERDs, which show robust antitumor activity in a variety of endocrine-sensitive and endocrine-resistant ER+ BC cell lines and patient-derived xenografts, including those harbouring *ESR1* mutations and provide some insights into their potential utility in combination with mTORC1 and CDK4/6 inhibitors.

Unique biology in invasive lobular breast cancer

Steffi Oesterreich. PhD; Professor

University of Pittsburgh

Invasive lobular breast cancer (ILC) is characterized by distinct etiological, pathological, and clinical features, yet the underlying biology is poorly understood. To extend the molecular determinants of ILC beyond the well-known loss of E-cadherin, we combined a plethora of in silico and wet-bench approaches, and comprehensively characterized ILC tumor models and clinical samples.

In support of recent data from The Cancer Genome Atlas on differences in mutations in critical ER co-factors between invasive lobular and ductal cancers, we have identified distinct ER signaling pathways, and mechanisms of endocrine resistance in ILC. These include the acquisition of unique ER target genes, activation of IGF and FGFR signaling, and changes in metabolic pathways. As part of our efforts to elucidate mediators of ILC progression, transcriptomic analysis of primary tissue from patients with and without disease recurrence identified potential genetic drivers, including Cortactin, an actin-binding protein with a known role in metastasis. To further identify genes that mediate the unique metastatic properties of ILC cells, we sequenced metastases to the ovary and the gastrointestinal tract, and uncovered pathways that might serve as critical vulnerabilities and therapeutic opportunities. In this lecture, I will review our progress, and discuss the potential clinical significance and implications of these findings.

Genetic aspects of lobular breast cancer

Professor Elinor Sawyer

Kings College London / Guy's Cancer Centre

Invasive lobular breast cancer (ILC) accounts for ~15% of invasive breast cancer and is commonly associated lobular carcinoma in situ (LCIS). Both ILC and LCIS have been shown to have higher familial risks than the more common ductal cancers and are more likely to be bilateral. However there is little data on the prevalence of the known high and moderate penetrance breast cancer predisposition genes in lobular breast cancer, with the exception of *CDH1*. In this talk I will present data on the frequency of germline mutations in *BRCA2, BRCA1, TP53, CHEK2, PALB2* and *CDH1* in sporadic ILC and LCIS. In addition, I will discuss the contribution of common single nucleotide polymorphisms to lobular breast cancer risk and show that many of the ER+ breast cancer predisposition loci also predispose to ILC, although there is some heterogeneity between ER+ lobular and ER+ IDC tumours.

Should lobular phenotype be considered when deciding treatments?

Prof Michael Kerin

Lambe Institute for Translational Research, NUI Galway & Galway University Hospital

Invasive lobular breast carcinoma (ILC), characterised by loss of E Cadherin, accounts for 10–15% of all breast cancers and is distinct from the more common invasive ductal carcinoma (IDC). Despite differences in screening efficacy, reduced chemo-sensitivity, altered patterns of and timing of metastasis, a patient with ILC tend to enter the same therapeutic algorithm as those with IDC. Contrary to convention, a diagnosis of ILC does not lead to excessive risks of contralateral breast cancer or nodal metastasis but has a distinct pattern of distant relapse.

The molecular era of breast cancer has led to the development of a subtype-based approach to management dictated by expression of oestrogen (ER), progesterone (PR) and Her2 receptors. A large majority of ILC are Luminal A and grade 2 and exhibit poor rates of complete pathological response to neoadjuvant chemotherapy. In addition, they are more likely to be mammographically occult and evident on MR mammography.

The utility of Oncotype dx in ILC remains unclear with emerging evidence that a) the scores are unique and b) pleomorphic lobular carcinoma (often Her2 positive) generate high scores. Only 5% of ILC is HER2 positive compared to 20–25% of IDC and this impacts Trastuzumab use.

From a surgery point of view, clinical decisions are based around tumour size and while the amount of breast tissue removed is often larger, therapeutic mammoplasty facilitates a conservative approach. In those with large Luminal A tumours, neoadjuvant endocrine therapy can lead to cytoreduction and improved breast conservation. With regard to adjuvant endocrine therapy, evidence from BIG 1-98 suggests that both Luminal A and Luminal B lobular carcinomas have improved disease-free survival with letrozole compared to tamoxifen.

ILC remains a distinct relevant breast cancer subtype which requires consideration in making patient-centred individualised decisions. Further prospective studies required to facilitate future evidence-based clinical decision making are necessary.

Communicating the potential benefits and harms of medical interventions

Professor Sir David Spiegelhalter

University of Cambridge

There is increasing acceptance that medical interventions should be the result of an informed choice, which means that potential benefits and harms need to be discussed in a way appropriate to a patient's wishes and needs. For breast cancer, this is reflected in the current leaflets that encourage women to weigh up the pros and cons of breast screening, and I shall look at the process by which these were developed. After diagnosis, prognostic calculators may be used in discussing possible adjuvant therapies, and I shall describe how a new front end to the PREDICT program is being developed using the principles of user-centred design, in which both medical professionals and patients contribute to both appearance and functionality. Finally, I shall touch on the additional communication challenges presented by genomic testing, both in healthy individuals and after diagnosis.

Who needs extended endocrine therapy?

Daniel F. Hayes, MD, FASCO, FACP, Stuart B. Padnos Professor of Breast Cancer Research

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Adjuvant endocrine therapy (ET)(tamoxifen or an aromatase inhibitor (AI)) for 5 years reduces the risk of distant recurrence and death in estrogen receptor (ER) positive, early-stage breast cancer[1]. Several studies have demonstrated that extended ET after 5 years of tamoxifen prevents later relapse and mortality[2–4], and recent reports suggest that five additional years of an AI after a preceding 2–5 years of an AI may further reduce risk[5,6].

These therapies have associated life-threatening or permanently life-changing toxicities, such as thrombosis and endometrial cancers (tamoxifen) or osteoporotic fractures and perhaps cardio/cerebrovascular disease (AIs). Both types of ET are associated with side effects that alter quality of life, such as hot flushes, sexual difficulties, and musculoskeletal symptoms.

Ideally, one would like to identify those patients with such a favorable prognosis that extended ET risks outweigh the benefits. Recently reported data from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) have provided accurate estimates of the risk of distant recurrence according to nodal status, size, and grade in women who were assigned to ET for 5 years and then to stop in prospective trials[7]. These data may be helpful to inform patients and their health care providers as they consider whether to continue ET or not.

Additionally, several single-trial correlative studies have suggested that multi-parameter, tissue-based assays, such as the 21-gene RS (OncotypeDX), the 12-gene RS (EndoPredict), the 2-gene assay (Breast Cancer Index), the PAM50-ROR (ProSigma), and the IHC4 tests may identify patients with a very low risk of late recurrence[8]. However, these studies have not been validated with sufficiently high levels of evidence to have clinical utility. Finally, identification of risk status in real time with liquid biopsies has gained substantial interest, but data to suggest they should be used are lacking.

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Management and follow-up of patients presenting with breast cancer in axillary lymph nodes and occult breast primary

Dr Andreas Makris

Mount Vernon Cancer Centre, Northwood, UK

1% of women presenting with breast cancer have isolated axillary lymph nodes with no detectable breast primary tumour. As would be expected from the low incidence, there are limited data (especially from prospective randomized trials) to guide treatment decisions, and management is often based on the analogous situation of patients presenting with breast tumours and involved axillary lymph nodes.

Breast MRI has demonstrated improved detection rates when compared to mammography and US. Further imaging with CT or PET-CT scans is used for staging to detect other metastases but may also show another primary. ER, PR, and HER2 are used to establish a breast origin and to guide systemic treatment.

Axillary dissection is routinely used. If the breast is not treated then about 50% of patients will relapse in the breast, and consequently the breast is treated either by mastectomy or radiotherapy (RT). As outcomes of women who have had mastectomy or breast conservation and RT are similar the latter approach is more appropriate. Additionally RT is used to the SCF and also to the chest wall after mastectomy.

With regards to adjuvant systemic therapy, this is routinely offered as would be the case for stage II/III breast cancers presenting with breast tumours. If chemotherapy (+anti-HER2 treatment for HER2+ tumours) is used, this treatment may be initiated prior to surgery, particularly as these patients often present with large axillary nodes. For women with ER+ tumours, endocrine therapy is routinely used.

With improving systemic treatments, including anti-HER2 agents such as trastuzumab and pertuzumab, there is a need for further studies to determine the optimal management of patients presenting with axillary metastases and occult primary tumours.

When is local surgery indicated in metastatic breast cancer?

Miss Nicola Roche

The Royal Marsden Hospital

Approximately 5% of newly diagnosed UK breast cancer patients present with Stage IV disease. There remains uncertainty as to the role of surgery for the primary tumour in this setting. Numerous retrospective case reports and their meta-analyses have suggested that surgery confers a survival benefit but selection bias is likely to have a significant role, in that patients that are operated on are fitter, younger, have lower volume disease, single site metastases or bone only metastases.

The primary tumour may act as site for further seeding of disease or may have an immuno-modulatory effect on distant disease sites.

Resection of the primary tumour in renal cell carcinoma has shown to confer an overall-survival (OS) advantage. There are currently seven randomised controlled trials (RCT) comparing surgery to the primary versus no surgery in stage IV breast cancer. Two have presented results and failed to demonstrate an overall-survival benefit in patients who had surgery to the primary tumour[1,2]. Similarly a registration study across 14 USA centres (TBCRC-013)[3] demonstrated no OS benefit of surgery to the primary tumour in the patient population who responded to initial systemic therapy. In patients who did not have surgery to the tumour, 2% of those responded to systemic therapy and 18% of those did not require surgery for local control.

Current evidence would suggest that outside of a trial, surgery to the primary breast tumour should be reserved to local disease control.

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What is the impact of MRI scanning on breast cancer treatment?

Prof. Dr. Ulrich Bick

Department of Radiology, Charité – Universitätsmedizin Berlin, Germany

If and when breast MRI should be used in women with newly diagnosed breast cancer is one of the most controversially discussed topics in breast imaging. Even though breast MRI is by far the most sensitive imaging test for the detection of malignant changes in the breast (both invasive and in situ) and MRI has clearly been shown to be superior to clinical exam, mammography, and ultrasound in defining the local extent of disease, conclusive evidence to support the routine use of breast MRI before initiation of breast cancer treatment is still lacking. The benefit of preoperative MRI will be largest in women with a high risk of multicentric or bilateral disease and in cases, where conventional imaging is impaired, e.g. through dense breast tissue. Histological confirmation of additional disease detected on MRI should be obtained whenever possible and good availability of MRI-guided interventions is a prerequisite for performing preoperative MRI's to avoid potential delays in treatment. Any decision whether to perform a preoperative MRI at all or whether to act on a new, often non-specific, additional finding on MRI in patients with newly diagnosed breast cancer has to be considered in the overall clinical context, as long-term prognosis of the patient is usually determined by the already known primary cancer.

Current and future indications for partial breast radiotherapy

Dr Charlotte Coles

CRUK Cambridge Centre, University of Cambridge

The scientific rationale for partial breast irradiation (PBI) is that the majority of breast tumours relapses occur close to the tumour bed of the original cancer. Therefore, the aim of PBI is to treat the region around the tumour bed as opposed to the whole breast, in patients at low risk of recurrence. The research hypothesis for PBI is there will be less long-term side effects, whilst maintaining excellent local tumour control.

Partial breast irradiation is not a new concept and several trials were carried out in the 1980s, but these showed an unacceptable high level of local relapse. This was probably due to suboptimal patient selection and older radiotherapy planning and treatment delivery techniques.

In the last decade, there has been a renaissance in PBI trials by virtue of newer methods of localisation and radiation treatment. This presentation will review the published reports to date of the following phase III trials:

- GEC-ESTRO (brachytherapy)
- IMPORT LOW (external beam)
- RAPID—interim results (external beam)
- ELIOT (intraoperative radiotherapy)
- TARGIT (intraoperative radiotherapy)

The presentation will also outline the study designs of the unpublished PBI trials due to be reported in the coming months and on-going research initiatives in this area. Finally, the question of which patients are likely to benefit most from PBI will be considered based on the evidence to date.

O-01

RADICAL phase Ib/IIa study of AZD4547 combined with anastrozole or letrozole in AI resistant ER+ breast cancer patients

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Background: Patients with metastatic ER+ breast cancer almost invariably experience disease progression whilst taking AIs. Fibroblast growth factor receptor inhibitors, e.g. AZD4547, can reverse endocrine resistance in breast cancer cells. Consequently, we designed the RADICAL trial to test safety and efficacy of AZD4547 combined with letrozole (L) or anastrozole (A).
Methods: Phase Ib demonstrated AZD4547 80 mg bd, 1 week on/one week off schedule in combination with L(2.5 mg)/A(1 mg) OD is safe and well tolerated.
Results: Enrolled patients previously received a median of 4 (range: 1–11) systemic therapies, including endocrine treatments with a median of 2 (range 1–6). Mean tumour size change at 12 and 28 weeks was 7% (95% CI − 4, 17%) and 8% (95% CI − 4, 20%), respectively. Clinical benefit assessed by partial response (PR) or stable disease (SD) occurred in 36.5% (1 PR and 18 SD) and 25% (2 PR and 11 SD) of patients at 12 and 28 weeks, respectively. Median progression-free survival was 3.1 months (95%CI 2.4–5.4).
Pharmacokinetic data showed no significant interactions. Subsequently, 52 patients progressing on these AIs were recruited, either continuing, or restarting their prior AI together with AZD4547. Primary endpoint was change in tumour size (RECIST v 1.1) at 12 weeks compared to baseline.

Most adverse events (AEs) were G1/2 (95.3%). 11 (21%) patients developed asymptomatic AZD4547-induced retinal pigment epithelial detachment, all resolved and 1 and 6 were able to continue on study medication at full and half dose, respectively. Among 34 G3/4 AEs, only six were probably/possibly related to AZD4547. Of 13 unrelated serious AEs, 2 were fatal.
Conclusions: Combined AZD4547 with L or A appears to be safe and shows anti-tumour activity in advanced ER+ patients resistant to these AIs. Development of a biomarker to select patients for this therapy will facilitate future studies.

O-02

Does immediate breast reconstruction delay the delivery of adjuvant treatment? The iBRA-2 prospective multicentre cohort study

The Breast Reconstruction Research Collaborative

The Breast Reconstruction Research Collaborative, Nationwide, United Kingdom

Introduction: Immediate breast reconstruction (IBR) is routinely offered to improve quality of life for women with breast cancer requiring a mastectomy, but there are concerns that more complex surgery may delay the delivery of adjuvant oncological treatments and compromise long-term oncological outcomes. High-quality evidence, however, is lacking. iBRA-2 is a national prospective multicentre cohort study that aimed to investigate the impact of IBR on the delivery of adjuvant therapy.
Methods: Breast and plastic surgery centres performing mastectomy with or without (±) IBR were invited to participate in the study through the trainee research collaborative network. All women undergoing mastectomy ± IBR for breast cancer between 1 July and 31 December 2016 were eligible for inclusion. Patient demographics, operative, oncological and complication data were collected. Time from last definitive cancer surgery to first adjuvant treatment for patients undergoing mastectomy ± IBR were compared to determine the impact that IBR has on the time of delivery of adjuvant therapy.
Results: 2548 patients were recruited from 76 centres of whom, 1016 (39.9%) underwent IBR. 675 (26.5%) patients received implant-based reconstruction; 105 (4.1%) pedicled-flaps and 228 (8.9%) free-flap reconstructions. Complications were experienced by 36.6% ($n = 932$) patients. There were no significant differences in complication rates between procedure types ($p = 0.12$), but patients undergoing IBR were significantly more likely to require re-admission ($p < 0.001$) or re-operation ($p < 0.0001$) for complications than undergoing mastectomy only. Adjuvant chemotherapy or radiotherapy was required by 1241 (48.7%) patients and no differences were seen in time to delivery of adjuvant therapy between patient groups.
Discussion: IBR is associated with a higher rate of complications requiring re-admission to hospital or re-operation compared to mastectomy alone but does not appear to significantly impact the time to delivery of adjuvant therapy. This study provides important information to guide patients and professionals making decisions regarding reconstructive surgery in the future.

O-03

Targeting IL1β-Wnt signalling prevents breast cancer colonisation in the bone microenvironment

Rachel Eyre[1], Denis Alferez[1], Angelica Santiago-Gomez[1], Kath Spence[1], James McConnell[1], Bruno Simoes[1], Claire Hart[1], Diane Lefley[2], Claudia Tulotta[2], Austin Gurney[3], Noel Clarke[1], Sacha Howell[1], Michael Brown[1], Andrew Sims[4], Gillian Farnie[5], Penelope Ottewell[2], Robert Clarke[1]

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Dissemination of tumour cells to bone marrow is an early event in breast cancer, however these cells may lie dormant in the bone environment for many years prior to eventual colonisation. Treatment for bone metastases is not curative, therefore new adjuvant therapies preventing disseminated cells from becoming metastatic lesions may be an effective therapeutic option to improve clinical outcomes.

There is evidence that cancer stem cells (CSCs) within breast tumours are the cells capable of metastasis; however, little is known about which bone marrow-derived factors support dormant CSC survival and eventual colonisation. Using in vitro culture of primary human bone marrow and patient-derived breast cancer cells, and in vivo metastasis models of human breast cancer cells implanted into mice, we investigated signalling pathways regulating CSC colony formation in bone.

We demonstrate that exposure to the bone microenvironment stimulates breast CSC colony formation in 15/17 patient-derived early breast cancers in vitro, and promotes a 3–4-fold increase in colony formation in breast cancer cells injected intra-femorally in vivo ($p < 0.05$). Further, we establish that IL1b secreted by human bone marrow induces breast CSC colony formation via intracellular NFkB signalling that induces Wnt secretion. Crucially, we show that inhibiting either IL1β (using an IL1β neutralising antibody or the IL1R antagonist Anakinra) or Wnt signalling (using Vantictumab, a therapeutic antibody which binds 5/10 Frizzled receptors), reverses induction of CSC activity by the bone marrow in vitro (Anakinra; $p < 0.0001$, Vantictumab; $p < 0.01$) and prevents spontaneous bone metastasis in vivo (IL1β neutralising antibody; $p < 0.02$, Vantictumab; $p < 0.01$).

These data indicate that IL-1β-Wnt inhibitors will prevent disseminated CSCs from forming metastatic colonies in bone, and represent an attractive adjuvant therapeutic opportunity in breast cancer. Drugs which target IL-1β (Anakinra and Canakinumab) are FDA-approved for other indications, and anti-Wnt treatments (Vantictumab) are in clinical trials in cancer, making this a viable therapeutic target in breast cancer patients.

O-04

Clinicopathological Significance of Heterogeneity of Tumour-Infiltrating Lymphocytes in Invasive Breast Cancer

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Background: Tumour-infiltrating lymphocytes (TILs) in breast cancer (BC) confer prognostic and predictive information. This study aims to assess the spatial and temporal heterogeneity of TILs and its relationship with subtypes of immune cells expression.
Material and method: Full-face tissue sections from a well-characterised BC series ($n = 273$) were examined for TILs heterogeneity (H&E) within primary tumours and the corresponding recurrent carcinomas. Intratumoural TILs heterogeneity was assessed using multiple tumour blocks ($n = 52$) representing different tumour areas. TILs density assessed using hot spots (HS) and stromal average (AV) in each of the stained sections. Association of TILs density with immune cell subtypes defined using immunohistochemistry (T cell markers; CD3, CD8, and FOXP3, B-cell markers; CD20 and histiocytic marker; CD68) was evaluated.
Result: Intratumoural TILs heterogeneity were not statistically significant between tumour areas. Primary tumour TILs were more heterogeneous than those in matched recurrent tumour ($p < 001$).TILs in primary BC were significantly associated with high-grade, poor prognosis Index, and triple-negative TNBC ($p < 05$). Higher TILs in primary TNBC was associated with longer BC–specific survival (BCSS) ($p = 0.046$) and distant metastasis-free interval ($p = 0.002$). CD3+, CD20+, and CD8+ cells associated with good outcome in TNBCSS ($p = 0.02$, $p = 0.037$, and $p = 0.005$, respectively). High expression of CD68+ and Foxp3+ was associated with poor outcome in non-TNBCSS ($p < 0.001$, $p = 0.010$). In recurrent BC, higher TILs density was associated with shorter post-recurrence BCSS ($p = 0.004$).
Conclusion: Although TILs were spatially heterogeneous, TILs assessment using one full-face section can reliably represent whole tumour TILs. AV-TILs in H&E slide including HS are reproducible in the clinical setting. TILs are not only associated with outcome in the primary tumour particularly in TNBC but also provide prognostic significance in the recurrent tumours.

O-05

Peri-operative Aromatase Inhibitor treatment in determining or predicting Long-term Outcome in Early Breast Cancer—the POETIC* Trial (CRUK/07/015)

Ian Smith

Royal Marsden Hospital, London, United Kingdom

Background: Experimental evidence (Fisher et al., 1989) and a small clinical trial (IMPACT), respectively, suggested peri-operative endocrine therapy (ET) may improve long-term disease-related outcome in patients undergoing primary surgery for ER positive (ER+) breast cancer (BC) and that tumor Ki67 levels after 2 weeks of peri-operative aromatase inhibitor (POAI) therapy might offer an effective way of predicting outcome and the need for additional adjuvant treatment. POETIC (*PeriOperative Endocrine Therapy-Individualising Care) is a phase-III randomized controlled trial designed to test these hypotheses and provide data to determine whether 2-week Ki67 improves prediction beyond that by baseline Ki67 of the group who have a higher risk of relapse in the first years after diagnosis in spite of best current standard of care.
Patients and methods: Postmenopausal patients with ER + BC were randomized 2:1 to either, POAI (center choice:
letrozole 2.5 mg or anastrozole 1 mg daily) for 14 days prior to and 14 days following surgery or no POAI (Control). Randomization was stratified by treating center; adjuvant treatment was per UK routine practice. Tissue samples were collected at baseline and surgery (FFPE) for blinded Ki67 testing. Primary endpoint was Time to Recurrence (TTR: time from randomization to loco-regional or distant recurrence or BC death). A secondary endpoint was Ki67 at baseline and after 2 weeks of AI.
Results: Between 2008 and 2014, 4480 patients (2976 AI, 1504 Control) were randomized from 130 UK centers. Median age was 67 (IQR 62-75), 18% had grade 3 tumors, 39% were node positive, and 61% had tumor size > 2 cm. For adjuvant ET, 314 patients (7.2%) received tamoxifen (Tam), 3695 (84.6%) an AI, 251 (5.7%) Tam changing to AI, and 109 (2.5%) changing from AI to Tam. On 8 August 2017, median follow-up was 60.7 months (IQR 49.5 to 72.2). 408/4480 (9.1%) patients have had a TTR event; 263 (8.8%) were allocated to POAI compared to 145 (9.6%) controls: HR 0.91 (95%CI 0.74, 1.12) Log-rank $p = 0.37$. Adjusted HR 0.91 (95%CI 0.74, 1.11). The relationship of Ki67 (baseline and after 2 weeks) with TTR in both the POAI & control groups will be presented for the overall ER+ population and HER2 defined sub-groups.
Discussion: There was no significant evidence that 4 weeks of POAI improved TTR compared with no POAI. POETIC will provide definitive evidence on the role of 2-week POAI-treated Ki67 to inform future practice and trials in terms of the potential to identify a group of patients for whom current standard of care appears insufficient in the few years post diagnosis.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

O-06

Increasing the dose density of adjuvant chemotherapy by shortening intervals between courses or by sequential drug administration significantly reduces breast cancer recurrence and mortality: an EBCTCG meta-analysis of 34,123 women in 25 randomised trials

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Background: Cytokinetic modelling suggests that increasing the dose density of cytotoxic therapy by shortening the intervals between courses, or by using sequential rather than concurrent treatment schedules may enhance efficacy. This EBCTCG meta-analysis brings together the evidence from 26 trials to clarify the balance of risks and benefits of dose-dense anthracycline and taxane chemotherapy.
Methods: Individual patient data were provided for 25 trials that included 94% (34,123/36,292) of women randomised in the 31 relevant trials. Primary outcomes were recurrence and breast cancer mortality analysed by standard log-rank methods.
Results: Highly significant reductions in disease recurrence [rate ratio (RR) 0.85 (95% CI 0.81–0.89), $p < 0.00{,}001$] were seen with dose-dense compared with standard chemotherapy. 10-year breast cancer mortality was 2.5% lower [19.4 vs. 21.9%: RR 0.87 (95% CI 0.82–0.92), $p < 0.00{,}001$], and overall-survival was also improved [RR 0.86 (95% CI 0.82–0.91), $p < 0.00001$]. Non-breast cancer mortality was if anything lower with dose-dense than standard chemotherapy [RR 0.83 (95% CI 0.73–0.95), $p = 0.008$]. The reductions in disease recurrence were similar in the seven trials that compared 2 weekly dose-dense chemotherapy versus the same chemotherapy given 3 weekly [rate ratio (RR) 0.83 (95% CI 0.76–0.91), $p = 0.00004$], the five trials of sequential versus concurrent taxane plus anthracycline chemotherapy [RR 0.88 (95% CI 0.75–0.91, $p = 0.002$)], and the six trials testing both shorter intervals and sequential administration [RR 0.83 (95% CI 0.75–0.91, $p = 0.0002$)]. The proportional reductions in recurrence with dose-dense chemotherapy were similar and highly significant (both $p < 0.00001$) in ER-positive and in ER-negative disease, and did not differ significantly by any other patient or tumour characteristics, including age, HER2 status, nodal status, tumour size, and grade.
Conclusion: Increasing the dose density of adjuvant chemotherapy reduces the risk of disease recurrence and death from breast cancer with no increase in deaths from other causes.

O-07

Integration of clinical variables for the prediction of late distant recurrence in patients with oestrogen receptor positive breast cancer treated with 5 years of endocrine therapy

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Abstract
Background: The prediction of late distant recurrence (DR) is an important clinical goal for managing women with hormone receptor positive disease who have reached the end of 5 years' endocrine treatment without recurrence. Molecular profiles have produced conflicting results for the prediction of late DR. Here, we develop and validate a simple clinicopathological tool [Clinical Treatment Score post-5 years (CTS5)] to estimate the residual risk of DR after 5 years' endocrine treatment, which should help in discussions with patients about the potential benefits or not of continued endocrine therapy.
Patients and methods: The ATAC dataset ($n = 4735$) of postmenopausal women with oestrogen receptor (ER)-positive breast cancer treated with 5 years' tamoxifen or anastrozole was used as a training cohort to establish a prognostic score for post-5-year risk of DR. CTS5 was based on five categories for nodal status, linear and quadratic terms for tumour size (capped at 30 mm), three categories for grade, and continuous age. The validity of the CTS5 was tested in the BIG1-98 dataset ($n = 6711$), which included postmenopausal women with ER-positive breast cancer treated with tamoxifen or letrozole (either monotherapy or sequential). Both cohorts included women who were alive and DR-free 5 years after randomization. Time to late DR, defined beginning at 5 years after ATAC or BIG 1-98 randomization, was the primary endpoint. Cox regression models estimated the prognostic performance of the CTS5. Hazard Ratios (HRs) are for a change of one Standard Deviation.
Results: The CTS5 model was a significant predictor for late DR in ATAC (HR 2.47 (95% CI 2.24–2.73) and performed better than the established 0–10 year CTS model (Cuzick et al., JCO, 2011). CTS5 was confirmed as highly predictive for late DR in the BIG1-98 validation cohort (HR 2.07 (1.88–2.28), $p < 0.001$). Of greatest importance was that CTS5 risk stratification, which defined in the training cohort as low (< 5% risk of DR during years 5–10), intermediate (5–10% risk), high (> 10% risk), identified 43% of the validation cohort as low risk, with an observed DR rate of 3.6% (95% CI 2.7–4.9) during years 5–10. Within nodal subgroups, 63% of node-negative were low risk with 3.9% (2.9–5.3) DR rate between years 5 and 10, and 24% having 1–3 nodes positive were low risk with 1.5% (0.5–3.8) DR rate between years 5 and10. Separation of intermediate-risk from high-risk categories was also shown in the training set but improvements in calibration seem necessary for clinical utility for that assessment.
Conclusion: The CTS5 is a simple tool based on information that is readily available to all clinicians. It was more accurate in its prediction of DR risk in years 5–10 than the published CTS model. CTS5 was validated as highly prognostic for late DR in the independent BIG 1–98 study. The algorithm identified a subgroup of women with either node-negative disease or 1–3 positive nodes as having less than 1% per year risk of DR who could be advised of the limited value of extended endocrine therapy.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

O-08

A phase III multicentre double blind randomised trial of celecoxib versus placebo in primary breast cancer patients (REACT—Randomised EuropeAn Celecoxib Trial)

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Background: Inhibition of COX-2 has been shown to attenuate the metastatic process in pre-clinical models of human breast cancer (BC). The primary aim of this study was to assess the effect of 2-year adjuvant therapy with the COX-2 inhibitor celecoxib compared with placebo in HER2−ve primary BC patients.

Patients and methods: Patients were randomised in a 2:1 ratio to receive celecoxib 400 mg once daily or placebo for 2 years. Patients had to have completely resected BC with prior local and systemic adjuvant treatment according to local practice. Concurrent radiotherapy was permitted and hormone receptor +ve patients received endocrine therapy according to local practice. Patients with HER2+ or node negative, T1, and grade 1 disease were excluded. Median age of patients was 55 years (IQR 49–63). 50% of patients had tumours > 2 cm; 42% were grade 3; 48% had node +ve disease. According to local assessment 73% were ER/PgR +ve. Primary endpoint was Disease-Free Survival (DFS), defined as time from randomisation to date of first event, with events contributing to analysis defined as recurrence (distant/local), new primary BC (ipsilateral/contralateral), and death. Secondary endpoints included Overall-Survival (OS), toxicity, cardiovascular mortality, and incidence of second primaries. Subgroup analysis by hormone receptor status was pre-planned. Survival endpoints are analysed using Cox proportional hazards and log-rank tests; restricted mean survival is used where proportional hazards do not hold.

Results: Between January 2007 and November 2012, 2639 patients were randomised (1763 celecoxib; 876 placebo) from 181 centres across the UK and Germany. On 13 April 2017, median follow-up was 60 months (IQR 48–72) with 428 DFS events reported. Unadjusted survival analysis results are presented below, with hazard ratio < 1 favouring celecoxib:

	5-year survival estimate (95% CI)	Hazard ratio (95% CI)	p value
DFS (all patients)			
Celecoxib	83% (81, 85)	1.02 (0.83–1.24)	0.88
Placebo	83% (80, 86)		
DFS within ER+			
Celecoxib	87% (85, 89)	0.89 (0.69–1.16)	0.40
Placebo	86% (83, 89)		
DFS within ER−			
Celecoxib	72% (68, 76)	1.17 (0.85–1.61)	0.33
Placebo	75% (69, 80)		
OS (all patients)			
Celecoxib	90% (88, 91)	0.97 (0.75–1.25)	0.81
Placebo	90% (88, 92)		

The interaction between ER status and treatment was not significant; $p = 0.36$

In the celecoxib and placebo groups, there were 17 and eight deaths, respectively, in patients who had not relapsed. These were due to cardiac ($n = 3$; 2) and other ($n = 14$; 6) in the celecoxib and placebo groups, respectively; none were GI-related. In total, 304 serious adverse events were observed in 265 patients (186/1763 celecoxib; 79/876 placebo). In the celecoxib and placebo groups, respectively, these were related to cardiac ($n = 12$; 7), GI ($n = 9$; 2), and other ($n = 193$; 81). Work is ongoing to determine whether a subset of ER+ patients whose primary tumours show the characteristics of a COX-2 signature receive greater benefit from celecoxib.

Conclusions: There is no benefit of celecoxib in the ITT population. Further exploratory studies focusing on the ER+ subpopulation are ongoing. Celecoxib treatment is not associated with significant toxicity when compared to placebo in this population of BC patients.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

O-09

Impact of a BRCA germline mutation on survival—Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH)

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Background: BRCA carrier breast cancers may have better, worse, or similar outcomes to non-carriers. To date there are no well-powered, prospective studies with adequate clinical data to address this important clinical question.
Methods: The Prospective Study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) recruited 3095 participants from UK oncology centres. Blood DNA was collected at recruitment, 2732 patients included in this analysis were aged 18–40 at breast cancer diagnosis and were analysed for BRCA1/2 mutations. Clinicopathological treatment and long-term outcome data were used to compare overall-survival (OS) and distant disease-free survival (DDFS) in all BRCA1/2 carriers and non-carriers in multivariable analyses (MVA). A pre-planned sub-group analysis examined OS and DDFS in patients presenting with hormone and HER2 receptor-negative cancer (TNBC).
Results: A pathogenic mutation was detected in 337/2732(12.3%) patients (200 BRCA1, 137 BRCA2). At a median follow-up of 8.2 years 649/2732 (27.4%), participants had died of breast cancer. There was no significant difference in OS or DDFS between BRCA1/2 mutation carriers and non-carriers in multivariable analyses (OS: HR 0.94; 95% CI 0.74–1.19; $p = 0.61$; DDFS:HR 0.99; 95% CI 0.79–1.24, $p = 0.93$). However in the TNBC sub-group analysis, patients with TNBC ($n = 558$) who were BRCA mutation carriers had significantly better OS at 2 years (HR 0.59 [95% CI 0.35–0.99], $p = 0.047$) but the HR for survival varied over time, initially in favour of carriers but by 10 years the HR was 1.98 (0.78–5.05, $p = 0.167$).
Conclusion: Overall-survival after young breast cancer in undiagnosed BRCA gene carriers is not significantly different from non-carriers after accounting for biology and treatment. The early survival advantage suggests a need for caution in interpreting treatment trials, the late disadvantage likely reflects second primary cancers.

O-10

Comprehensive molecular characterisation of TNBCs expressing *HORMAD1*, a driver of homologous recombination deficiency

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Complex compositions of structural and single nucleotide variations are found in genomes of sporadic triple-negative breast cancers (TNBCs) and germline *BRCA1/2* mutation carriers. We recently identified *HORMAD1*, a Cancer/Testis (CT) antigen normally expressed in meiotic cells, as a novel driver of increased allelic imbalance alterations in TNBCs. In TNBC cell line models, HORMAD1 inhibits homologous recombination (HR), thereby increasing reliance on error-prone DNA damage repair mechanisms. To further explore the influence of *HORMAD1*, we interrogated transcriptomic and genomic profiles of *HORMAD1*-positive TNBCs.

Across 8 TNBC cohorts ($n = 1112$), including three clinical trials, *HORMAD1* expression was consistently bimodal. While in seven cohorts ($n = 873$) over 50% of the samples expressed *HORMAD1*, a reduced prevalence (37%) was observed in those developing metastasis. *HORMAD1*-positive TNBCs were enriched for PAM50 basal-like, IntClust 10, and the basal-like 1 TNBCtype-4 subtypes. Single nucleotide variations in *PIK3CA*, *NCOR2*, *KMT2D*, and *CECR2* were exclusive to *HORMAD1*-negative TNBCs. In *HORMAD1*-positive TNBCs, a higher transcriptional activity in genes involved in HR and mismatch repair was observed, consistent with its role in meiosis and DNA damage repair. Moreover, these TNBCs exhibited elevated levels of *CXCR3*, *CXCL10*, *TNFSF13B*, and *TMEM173*, highlighting a potential association with the STING pathway, in line with *HORMAD1* being a CT antigen. Acute *HORMAD1* expression in TNBC cell line models affected immune-associated genes (*CCL5*, *STAT3*, *CXCL2*, *TSC22D*), those involved in ubiquitination (*HUWE1*, *UBA1*), and DNA damage repair (*DDIT4*, *DDIT3*). Whole genome sequence analysis of TNBCs from TCGA and ICGC revealed an increase in the number of base substitutions, as well as the prevalence of mutational signature 3 and rearrangement signature five in *HORMAD1*-positive TNBCs.

Extensive molecular characterisation of *HORMAD1-p*ositive TNBCs provides a rationale for exploring novel therapeutic strategies including targeting DNA damage repair, and immunotherapy.

O-11

Comparative transcriptomic analysis of long-term neoadjuvant letrozole-treated dormant and acquired resistant tumours using sequential clinical samples

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Aim: To characterise genome-wide expression profiles of dormant and resistant breast cancer specimens receiving extended (4–45 months) neoadjuvant letrozole.
Patients and methods: Sequential patient-matched clinical samples treated with extended letrozole were used to model clinical breast cancer dormancy and acquired resistance. Patients with > 40% initial decrease in tumour size by 3 months of letrozole treatment and with a stable size by the latest biopsy were classified as "dormant" whereas those exhibiting progression following an initial > 40% decrease were classified as "acquired resistant" based upon change in tumour size by ultrasound. Expression analysis was performed using Illumina BeadChips and analysis performed using R and BioConductor packages.

Results: A total of 167 patient-matched samples from 'dormant' ($n = 42$) and 'acquired resistant' ($n = 20$) patients were compared using unpaired Rank Product analysis (FDR, 1%) at three time points: pre-treatment (< 0 days), early-on treatment (0-120 days), and after long-term (extended, > 120 days) neoadjuvant letrozole treatment. A total of 836 genes were significantly differentially regulated (525 down, 311 up) between long-term treated samples. Functional analysis (ReactomePA) revealed an enrichment for several cancer-related pathways, including extracellular matrix (ECM) organisation, ECM degradation, and DNA methylation. A subset of the genes including histone genes significantly separated 12 (out of 20) acquired resistant from dormant tumours after long-term treatment. Separation was also apparent early-on time point suggesting that these genes may be used to predict acquired resistance in a subset of patients within first 4 months of neoadjuvant treatment.
Conclusion: This is the first patient-matched gene expression study to look at long-term aromatase inhibitor-induced dormancy and acquired resistance in breast cancer. Our results may help to characterise extended growth suppression and escape from dormancy in letrozole-treated breast cancer cells.
This work was supported by Marie Skłodowska-Curie Individual Fellowship [H2020-MSCA-IF, 658170] and Welcome Trust Institutional Fund (ISSF3) to CS and AHS, Breast Cancer Now to AHS.

O-12

Finding determinants of PARP inhibitor sensitivity using genome-wide and focused CRISPR screens

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We are using genomic approaches to study the mechanism of action of PARP inhibitors. Experimental study of potential resistance mechanisms can inform the ongoing clinical development of these drugs, as well as reveal new aspects of PARP function in normal and homologous recombination deficient cells.

To investigate mechanisms of PARP inhibitor cytotoxicity, we carried out genome-wide screens for mutants resistant to the potent PARP inhibitor talazoparib (BMN 673) in mouse ES cells and human breast cancer cells. Many PARP inhibitor-resistant clones had loss-of-function *Parp1* mutations as expected. However, we also isolated a point mutation affecting a single amino acid in the Parp1 DNA binding domain. This mutant encodes a stable Parp1 protein that cannot bind DNA and does not become trapped in the presence of inhibitors. Thus the CRISPR screen implicated PARP1 DNA binding directly in determination of PARP inhibitor cytotoxicity and was extremely informative about the mechanism of action compared to conventional loss-of-function mutagenesis.

We extended this approach by synthesising a high-density focused sgRNA library targeting only *PARP1*. We developed a reporter cell line that allows us to selectively isolate in-frame mutations that preserve PARP1 protein expression. By deep-sequencing mutagenised and appropriately selected cells, we identified a series of subtle mutations in PARP1 that result in PARP inhibitor resistance, giving us a detailed insight into structure–function relationships in PARP1. Among these, we found mutants that display trapping despite conferring PARP inhibitor resistance, suggesting that PARP trapping is not sufficient for cytotoxicity. Mutations that confer resistance are restricted to DNA binding domains and a network of residues in the WGR and helical domains of PARP1 that may be involved in intramolecular activation of PARP1 upon DNA binding and thus affect trapping. Our experiments also show that these mutations can be tolerated in the context of some clinical *BRCA1* mutations and therefore may represent a clinical mechanism of PARP inhibitor resistance.

O-13

The LORIS Trial: Randomising patients with low- or low intermediate-grade Ductal Carcinoma In Situ (DCIS) to surgery or active monitoring

Matthew Wallis[1], John Bartlett[2], Lucinda Billingham[3], Sarah Bowden[3], Cassandra Brookes[4], David Dodwell[5], Andrew Evans[6], Patricia Fairbrother[7], Lesley Fallowfield[8], Claire Gaunt[3], Andrew Hanby[9], Valerie Jenkins[8], Lucy Matthews[8], Sarah Pinder[10], Sarah Pirrie[3], Daniel Rea[3], Malcolm Reed[11], Tracy Roberts[3], Jeremy Thomas[12], Margaret Wilcox[7], Jennie Young[3]

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Background: Overdiagnosis and thus overtreatment of screen-detected DCIS and invasive cancer is a recognised issue. The LORIS trial addresses the possible overtreatment of low- and low/intermediate-grade screen-detected (low-risk) DCIS
Trial design: LORIS is a phase III, multicentre, 2-arm, non-inferiority study, in patients with low-risk DCIS confirmed by central pathology review. Patients are randomised between standard surgery and active monitoring with annual mammography.
Key eligibility criteria:

(1) Screen-detected or incidental microcalcification (mass lesion clinically or on imaging proven to be benign)
(2) Low-risk DCIS on large-volume vacuum-assisted biopsy, confirmed by central pathology review
(3) Fit for surgery
(4) No previous breast cancer or ipsilateral DCIS diagnosis

Aims: LORIS will establish whether patients with newly diagnosed low-risk DCIS can safely avoid surgery without detriment to their physical and psychological well-being and whether patients who require surgery can be identified by pathological and radiological means.
Primary endpoint: Ipsilateral invasive breast cancer-free survival.
Secondary endpoints: Overall-survival; mastectomy rate; time to mastectomy; time to surgery; patient-reported outcomes; health resource utilisation; and assessment of predictive biomarkers. A digital image repository and tumour bank are collected.
Statistical methods: Patients will be randomised to test the null hypothesis that active monitoring of women diagnosed with low-risk DCIS is not non-inferior in terms of ipsilateral invasive breast cancer-free survival (iiBCFS) compared to treatment with surgery. The iiBCFS rate is assumed to be 97.5% in the surgery arm at 5 years,

utilising an 80% power with a 1-sided, 5% alpha to exclude a difference of more than 2.5% in the active monitoring arm requiring 932 patients.

Progress: The feasibility phase is complete. 47 UK centres are now open to accrual from a target of 60. The web-based central pathology review is functioning efficiently, with a one-week maximum turn around. Monthly dial-in, topic-focused teleconferences have proven very successful. Randomisations are currently approximately 81% of target.

Contact Information: Email the LORIS Trial Office on LORIS@trials.bham.ac.uk

O-14

The plasmaMATCH Trial: A multiple parallel cohort, open-label, multi-centre phase-II clinical trial of ctDNA screening to direct targeted therapies in patients with advanced breast cancer (CRUK/15/010).

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Introduction: Circulating tumour DNA (ctDNA) is found in the plasma of over 90% of patients with advanced breast cancer (BC). Screening for the presence of mutations in ctDNA provides a current assessment of the genetic profile of the patient's recurrent BC. The plasmaMATCH trial is designed to assess the potential of ctDNA screening to direct targeted therapies in patients with advanced breast cancer.

Methods: plasmaMATCH is a multi-centre phase-IIa umbrella trial platform of ctDNA screening and a therapeutic trial. The study will screen 1000 women with advanced breast cancer, who have received prior systemic treatment in the advanced setting, with digital PCR ctDNA assays for hotspot mutations in *ESR1*, *HER2*, *AKT1*, and *PIK3CA*, with *HER2* copy number assessment, in a central laboratory. The study will recruit from up to 50 sites in the UK. Patients with mutations identified will enter the matching treatment cohort, *ESR1*—extended dose fulvestrant 500 mg every two weeks, *HER2*—neratinib +/− fulvestrant, *AKT1*—AZD5363 +/− fulvestrant.

Mutation prevalence is presented with corresponding exact 95% confidence intervals (CIs) both overall and excluding 14 patients who were known to have mutations from a prior screening program. Patients with more than one mutation are included once in each relevant row.

Results: We report the results of prospective ctDNA mutation testing in the first 92 patients. plasmaMATCH opened to recruitment on 15/12/2016. As of 08/06/2017, 120 patients have been registered for ctDNA screening from seven UK centres, of which 92 have ctDNA screening results available (data accumulating, updated figures will be presented):

Mutation	Prevalence (95% CI)	Prevalence excluding 14 patients with known mutations (95% CI)
ESR1	34/92:37% (27–48%)	26/78:33% (23–45%)
HER2	5/90:6% (2–12%)	2/76:3% (0–9%)
AKT1	7/92:8% (3–15%)	4/78:5% (1–13%)
PIK3CA[a]	22/92:24% (16–34%)	21/78:27% (18–38%)

[a] No corresponding plasmaMATCH treatment cohort

14 patients had more than one mutation detected (10 *ESR1*+ *PIK3CA*, 3 *ESR1*+ *AKT1*, 1 *ESR1*+ *HER2*+ *AKT1*). ctDNA results were reported in a median of 8 working days.

Of the 40 patients with one or more actionable mutation, 15 have entered a cohort, 16 are being screened for entry into a cohort, five are currently receiving further systemic treatment prior to cohort entry, and four will not enter a cohort. One additional patient has entered a treatment cohort on the basis of a mutation detected in an alternative tumour sequencing initiative.

Conclusions: plasmaMATCH ctDNA demonstrates the feasibility and accuracy of ctDNA testing as a screening tool for patients with advanced BC, with a high rate of subsequent recruitment into matching therapeutic trials.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

CLINICAL BIOMARKER

P1.1

Predictive biomarkers for endocrine therapy: Retrospective study in Tamoxifen and Exemestane adjuvant multinational trial

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Background: Aromatase inhibitors improve disease-free survival compared with tamoxifen in postmenopausal women with hormone-receptor-positive breast cancer. The Tamoxifen and Exemestane Adjuvant Multinational (TEAM) trial compared exemestane monotherapy versus sequential therapy of tamoxifen followed by exemestane. The trial failed to show a significant difference between treatment arms. A robust translational program was established to investigate predictive biomarkers.
Methods: A TMA was retrospectively constructed using a subset of patient tissue from the TEAM trial ($n = 4631/9766$). Immunohistochemistry was performed for biomarkers, classed into three groups: MAPK pathway, NF-kappa B pathway, and ER phosphorylation. Expression was analysed for association with relapse-free survival (RFS) at 2.5 and 10 years and treatment regimen.
Results: On univariate analysis, ER^{167} (HR 0.71 95% CI 0.59–0.85, $p < 0.001$), IKKα (HR 0.74 95% CI 0.60–0.92, $p = 0.005$), $Raf\text{-}1^{338}$ (HR 0.64 95% CI 0.52–0.80, $p < 0.001$), and p44/42 $MAPK^{202/204}$ (HR 0.77 95% CI 0.64–0.92, $p = 0.004$) were significantly associated with improved RFS at 10 years in patients receiving sequential therapy. Associations were strengthened when IKKα, $Raf\text{-}1^{338}$, and ER^{167} were combined into a cumulative prognostic score (HR 0.64 95% CI 0.52–0.77, $p < 0.001$). Patients with an all negative IKKα, $Raf\text{-}1^{338}$, and ER^{167} score favoured exemestane monotherapy (OR 0.56 95% CI 0.35–0.90). On multivariate analysis, the IKKα, $Raf\text{-}1^{338}$, and ER^{167} scores ($p = 0.001$) were independent prognostic factors for RFS at 10 years in patients receiving sequential therapy.
Conclusions: The IKKα, $Raf\text{-}1^{338}$, and ER^{167} scores are independent predictive biomarkers for lower recurrence on sequential therapy. Negative expression may further offer predictive value for exemestane monotherapy.

P1.2

Audit of Oncotype Dx in the North East—can we improve our practice?

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Introduction: Breast cancer affects one in 8 women in their lifetime. Adjuvant chemotherapy is given to a significant proportion of women with early breast cancer. Oncotype Dx is a 21-gene assay looking at inherent tumour biology and is predictive of benefit from chemotherapy in patients with ER+ve, HER2-negative, and node-negative early breast cancer.
Aims

(1) Evaluate time from surgery to oncotype request date
(2) Evaluate time from surgery to adjuvant radiotherapy in patients not requiring chemotherapy
(3) Assess chemotherapy use in patients with intermediate-risk recurrence scores (RS 18–30)

Methods: Retrospective review of patients treated with surgery for early breast cancer at two regional breast screening centres between March 2015 and August 2016:
Centre 1—Oncotype Dx requested from post-surgical multidisciplinary team meeting and patients with low-risk recurrence scores diverted to radiotherapy one-stop clinics.

Centre 2—Oncotype Dx requested at first oncology appointment, second appointment made with results with subsequent referrals to radiotherapy clinics if no chemotherapy is planned.
Results: 85 patients—Centre 1$n = 51$, Centre 2$n = 34$
Median number of days from surgery to oncotype request date: 13 days Centre 1 versus 32.5 days Centre 2

Median number of days from surgery to adjuvant radiotherapy start date: 67.5 days Centre 1 versus 82 days Centre 2
$n = 29$ with intermediate-risk recurrence score: 32% received chemotherapy in the RS 18–25 group versus 50% in the RS 26–30 group.
Discussion: Oncotype Dx guides adjuvant chemotherapy decisions in patients with ER+ve, HER2−ve, and node−ve early breast cancer. This is discussed and offered to women in the two centres at different times using local protocols. Timings of adjuvant treatment are influenced by Oncotype Dx. We recommend standardising this process and organising the test at the earliest opportunity.
Chemotherapy offered and accepted by patients in the intermediate-risk group varies, with higher recurrence scores associated with higher use of adjuvant chemotherapy.

P1.3

Independent validation of the NHS PREDICT breast cancer prognostication and treatment benefit prediction tool using the Scottish cancer registry

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Objective: NHS PREDICT is an online prognostication and treatment benefit estimation tool for patients with early breast cancer. Originally launched in 2010, the underlying algorithm has recently been updated to include new prognostic markers and refined predictions. This study reports a validation of the recent version (version 2). High-quality data collected routinely in Scotland allow a broader assessment of external validity than previous studies.
Methods: Patient level data were extracted from the Scottish Cancer Registry. All records in a diagnosis of primary invasive breast cancer between January 2001 and December 2015 were included. Prognostic scores for breast cancer were estimated using NHS PREDICT version 2. Statistical discrimination was assessed by the area under the

receiver-operator curve (AUC). Calibration was assessed by comparing predicted deaths to observed deaths across relevant subgroups. Sensitivity analyses assessed the influence of sample selection rules and missing data. Analyses were repeated for all-cause mortality and breast cancer-specific mortality.
Results: In total, 30,061 eligible cases were selected from 61,437 individual records extracted from the registry. Discrimination was similar to that reported in the derivation sample; AUC for all-cause mortality: ER-positive 0.80 and ER-negative 0.76. Calibration was comparable with that reported for the validation sample. Predicted mortality rates were within 5% of observed mortality rates in all subgroups except those with tumour size greater than 50 mm.
Conclusion: Good discrimination and calibration was demonstrated in the Scottish population. These results improve upon the precision and generalisability of previous validation studies. The sample included the broadest possible set of cases, was of a large size, had a longer follow-up period than previous studies, and included more recent cases. Clinicians and patients can have confidence in NHS Predict as a reliable decision aid in Scotland.

P1.4

The impact of intrinsic subtypes and molecular features on aromatase inhibitor induced reduction of proliferation marker of Ki67 in primary ER+ breast cancer: a POETIC study (CRUK/07/015)

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Background: Neoadjuvant endocrine therapy (NAE) is often a good option for postmenopausal (PM) women with estrogen receptor-positive (ER+) breast cancers (BC). Fall in Ki67 is widely accepted as valid for predicting favorable tumor response to NAE and improved outcome. We report our planned correlative study to investigate if intrinsic subtype impacts on Ki67 changes (ΔKi67) as measured by immunohistochemistry. We also explored the correlation of several ER+ BC relevant molecular features at baseline (B) with ΔKi67.
Patients and methods: POETIC is a phase-III, randomized 2:1 study for 4486 PM patients with ER+ BC to determine whether peri-operative aromatase inhibitor (AI) followed by standard adjuvant therapy improves outcome compared with standard adjuvant therapy alone. The proliferation rate was estimated as percentage (%) of cancer cells staining for Ki67. Primary biological endpoint was defined as two-week (2wk) change in Ki67 (2wkΔKi67): ln[(2wk Ki67 + 0.1)/(B Ki67 + 0.1)]. Secondary endpoint: "responders," was % change of Ki67 defined as (2wk Ki67—B Ki67) *100/B Ki67. "Responder" was defined as follows: reduction $< 50\%$ as poor responder (PR), 50-75% intermediate (IR), and $> 75\%$ as good responder (GR).
Human whole genome expression (GE) BeadChips (Illumina, USA) were performed. Data were obtained from 137 paired samples from the treatment group (T) and 49 pairs from the control (C) group with GE data passing quality check and baseline Ki67 $^3 5\%$ to minimize the impact of extreme values based on proportional ΔKi67. Intrinsic subtype and risk of recurrence (ROR) groups were calculated using PAM50. GE scores from Oncotype Dx, MammaPrint, p53 Mutation/wildtype (Troester 2006), ER+ early response (ERE) (Hatzis 2011), estrogen-regulated genes subtypes (Oh 2006), and markers for 23 different immune cell types (Bindea 2013) were calculated. Associations of GE scores to endpoints of response were determined by Spearman correlation and χ^2 tests. Bonferroni correction was used to control the error rate with $p < 0.001$ deemed significant.
Results: At B of the 137 paired T, 64% were Luminal A (LumA), 22% Luminal B (LumB), 9% as HER-2 enriched (HER2-E), 2% as Basal-like (BLBC), and 3% as Normal-like. Subtypes at B were associated with response, with LumA showing the biggest reduction of Ki67 ($p = 0.00012$) and GR. All GE, except ERE, correlated significantly with 2wkΔKi67 and response: higher risk groups associated with lowest reduction rate. None of immune cell types correlated with 2wkΔKi67, except that tumors enriched with T-helper 1 cell type were associated with PR ($p < 0.000001$).
Comparing subtypes between time points (see table), 85% of LumB and 42% of HER2-E were assigned instead as LumA at 2wk regardless of response. Of 15 ROR high-risk at B, only 33% were assigned instead as low-risk at 2wk.
Conclusion: Both LumA and LumB are endocrine sensitive. A fall of Ki67 was observed in most tumors. Most tumors estimated as high-risk by molecular profiling showed less response and most remained moderate risk of recurrence on endocrine therapy. Whether molecular profiling at 2wk after starting AI predicts for long-term outcome in PM women with ER+ better than at diagnosis will need to be determined.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P1.5

EA2Clin: A Novel Immunohistochemical Prognostic and Predictive test for patients with Estrogen Receptor-Positive Breast Cancer

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Background: The majority of patients with early-stage estrogen receptor-positive (ER+) breast cancer (BC) are treated with adjuvant endocrine therapy (ET) after primary surgery to reduce the risk of recurrence. A variety of tests are available to predict outcomes on ET but most require gene-level measurements and are expensive. Recently, we developed an immunohistochemistry (IHC)-based test (EA2Clin) using levels of pre-treatment IL6ST together with clinical variables and on-treatment proliferation. The aim was to validate this test in cohorts of both pre- and post-menopausal women treated with two weeks of a variety of endocrine treatments (tamoxifen, fulvestrant, or an aromatase inhibitor) prior to surgery.
Methods: The cohorts are (A) 186 post-menopausal women (PMW) with ER+ BC treated with at least 2 weeks of preoperative or neoadjuvant letrozole or anastrozole, then surgery followed by adjuvant letrozole ($n = 132$) or tamoxifen ($n = 54$); (B) 51 pre-menopausal women (preMW) with ER + BC treated with 2 weeks of either neoadjuvant tamoxifen ($n = 24$) or one 750 mg dose of faslodex ($n = 27$), then surgery followed by adjuvant tamoxifen. The median follow-up was 5.4 years for cohort A and 10.2 years for

cohort B. IHC analysis was performed using a Leica BOND III Autostainer and the EA2Clin algorithm was used to stratify patients in binary high- or low-risk groups.
Results: In the cohort of PMW, EA2Clin was highly significantly associated with both recurrence-free survival (RFS) ($p < 0.0001$, HR 13.26, 95% CI 5.59–13.46) and breast cancer-specific survival (BCSS) ($p < 0.0001$, HR 12.93, 95% CI 4.43–37.72). The 5- and 10-year actuarial recurrence rates were 7/22 and 46/73% for the low- and high-risk groups, respectively. The actuarial breast cancer-related death rate for the low-risk group was 5% at both 5 and 10 years, whereas for the high-risk group it was 33/38%. Confounding factors were not found to be significant.
In the cohort of preMW, our test was significantly associated with both RFS ($p = 0.002$, HR 5.71, 95%CI 1.91–17.05) and BCSS ($p = 0.016$, HR 4.81, 95% CI 1.34–17.26). The 5- and 10-year actuarial recurrence rates were 12/29% and 27/77% for the low- and high-risk groups, respectively. The 5- and 10-year actuarial breast cancer-related death rates were 7/19% and 9/58% for low- and high-risk groups, respectively.
Discussion: This study has validated EA2Clin as the first predictive tool to incorporate clinical data with pre and on-treatment immunohistochemical biomarkers to predict accurately the outcome of patients with ER-positive breast cancer treated with adjuvant ET.

- This test predicts both RFS and BCSS in pre- and PMW treated with a variety of endocrine agents.
- Because this test incorporates clinical variables with simple IHC, it can be performed locally in any pathology lab.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P1.6

AZD5363, an AKT inhibitor, significantly inhibits key biomarkers of the AKT pathway and Ki67, in a randomized, placebo- controlled study (STAKT) in human breast cancers

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Introduction: AKT is an important intracellular control point. Mutations in *PIK3CA, AKT* and *PTEN* are prevalent in estrogen receptor-positive (ER+) breast cancer (BC) and have been implicated in resistance to endocrine therapies. AZD5363 is an inhibitor of AKT 1, 2, and 3.
Methods: STAKT is a multi-center, ethically approved, two-stage, double-blind, randomized, placebo-controlled, biomarker 'window-of-opportunity' trial in women with newly diagnosed, previously untreated ER+ BC. Stage 1 assessed AZD5363 at a dose of 480 mg bd p.o. versus placebo. Primary endpoint markers were pPRAS40, and pGSK3β and Ki67 assessed by immunohistochemistry: changes (both absolute and %) between biopsies compared between the two groups.
Results: 28/36 patients were evaluable with patient & tumor characteristics.
Total PRAS40 H-scores changed (versus baseline) by -83.8 (absolute reduction) and − 50.2 (% reduction) (both $p < 0.0001$). Cytoplasmic PRAS40 changes were − 90.0 and − 55.8 for absolute and % reduction, respectively (both $p < 0.0001$).

pGSK3β H-scores changes were: Total − 55.3 and − 39.0 for absolute and % reduction, respectively (both $p = 0.006$). Cytoplasmic − 53.6 and − 39.2 for absolute and %, respectively (both $p = 0.006$)

Nuclear staining for Ki67 showed − 9.6 absolute ($p = 0.031$) and − 29.4% reduction ($p = 0.052$).
Conclusions

- AZD5363 for 4.5 days caused highly significant falls in pGSK3β and pPRAS40.
- AZD53643 also caused a significant decline in Ki67 even after only 4.5 days of drug. This is one of the shortest 'window'-studies to report such an early effect on proliferation.
- Placebo-controlled 'window' studies of this short duration can provide important evidence of the therapeutic potential early in a drug's development.

P1.7

Change in Ki67 during neoadjuvant chemotherapy and its relationship to residual Ki67 and pathologic complete response

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Research objectives: To examine the hypothesis that the change in Ki67 following short-term (2/3 weeks') exposure to chemotherapy would be predictive of the overall change in Ki67 value at end of treatment or of pathologic complete response (pCR).
Background: Ki67 is a nuclear antigen that is widely used as a marker of proliferation. Studies looking at its expression in samples taken before and after neoadjuvant chemotherapy (NCT) have found it to be a much stronger prognostic factor when measured at the end of NCT. If changes earlier in NCT predicted for end of treatment values, this might allow change of management at an early stage, where it is required.
Materials and Methods: Biopsies were collected at baseline (B), following one-cycle (C), and at end of treatment (surgery, S) from patients with early locally advanced breast cancer scheduled to receive NCT. Ki67 was assessed by manual scoring in immunohistochemically stained sections (cases having pCR at surgery were given a Ki67 value of 0%).
Results: Seventy-two patients had a Ki67 result at all three time points, (including 23 with histologically confirmed pCR at surgery). The median change in Ki67 between B and C was − 3.9%, between B and S it was -23.4%. Significant correlation between fold-changes B-C and B-S was seen in the whole population ($r = 0.319$, $p = 0.006$). When stratified by ER and HER2 status, significant correlation in these two measures was seen only in ER-positive, HER2-negative patients ($n = 32$, $r = 0.392$, $p = 0.027$). Change in Ki67 from B to C was not associated with pCR at surgery (median − 3.8% in no-pCR group, − 6.9% in pCR-group; $p = 0.257$).

Conclusions: The change in Ki67 expression following short-term exposure to NCT was correlated with the Ki67 change between baseline and surgery but this correlation was insufficiently close for the early change to be a useful predictive measure.

P1.8

Focal Adhesion Kinase and Aldehyde Dehydrogenase 1 are prognostic cancer stem cell markers in invasive ductal carcinoma

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Background: Breast cancers exhibit a hierarchy whereby cancer stem cells (CSC) are treatment resistant and self-renew to initiate tumour recurrence. Accurate identification of CSCs is problematic. Aldehyde dehydrogenase 1 (ALDH1) is an accepted breast CSC marker. Integrin alpha-6 (ITGA6), a stem cell marker in normal breast, is proposed as a CSC marker. Focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase is believed to play a role in CSC self-renewal and treatment resistance.
We aimed to correlate FAK expression with CSC markers and patient outcome in tissue microarrays (TMAs) using immunohistochemistry (IHC).
Methods: IHC was used to detect expression of phosphorylated FAK (pFAK), total FAK (tFAK), ALDH1 and ITGA6 in a cohort of 244 invasive ductal carcinomas (IDC) in TMAs and correlated with clinicopathologic characteristics. ITGA6 expression was assessed in relation to patients with a known mammosphere forming efficiency (%MFE), a functional CSC assay, and in a group of patients treated with lapatinib alone or a combination of lapatinib and trastuzumab. Association with recurrence was assessed using χ^2 tests and multivariable Cox regression.
Results: Reduction in %MFE was observed following siRNA FAK knockdown in SUM159 and MDA-MB-231 breast cancer cell lines ($p < 0.05$). Increased tFAK expression was associated with poor disease-free survival in a multivariable analysis (HR 1.93, 95% CI 1.1–3.3, $p = 0.013$). High epithelial ALDH1 expression was associated with recurrence ($p = 0.018$). ITGA6 expression correlated with %MFE in non-invasive breast cancer samples ($\rho = 0.653$, $p = 0.029$, $n = 11$) and was reduced following treatment with anti-HER2 agents ($p = 0.002$, $n = 5$) matching a reduction in Ki67.
Conclusion: tFAK and ALDH1 are independent prognostic factors and identify patients who would potentially benefit from FAK-targeting CSC inhibitors. Reduction in CSC self-renewal following siRNA FAK knockdown establishes a functional role for FAK in CSCs. Correlation of IHC markers with an in vitro CSC marker assay supports the use of ITGA6 as a breast CSC marker for endpoints in clinical trials.

P1.9

A retrospective cohort study of the response to neoadjuvant chemotherapy in oestrogen receptor-positive and oestrogen receptor-negative breast cancers

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Background: Neoadjuvant chemotherapy is used to treat locally advanced breast cancers and aims to enable breast conservation surgery. There is some debate in whether oestrogen receptor (ER)-positive breast cancers benefit equally to NACT compared to oestrogen receptor-negative breast cancers, with regards to achieving a complete/partial response. This retrospective cohort study compared both breast cancer subtypes and their response to chemotherapy before surgery.
Methods: One hundred and forty-three patients out of 195 patients that received NACT from 2007 to 2015 at the Royal Devon and Exeter (RD&E) hospital were included in this study. Of these 143 patients, there were 91 ER-positive and 52 ER-negative breast cancer patients. Patient identifiers of those included were provided by Dendrite®, data were then collected from these patients via the clinical data management (CDM) database of the RD&E hospital. Explicit responses and calculated responses (from sizes before and after surgery) were recorded.
Results: ER-negative patients achieved a higher proportion of pCR compared to ER positive cancers, OR 4.45, CI 2.26–8.75, ($p = <0.0001$). Oestrogen receptor-positive cancers had a higher proportion of NR (OR 4.95, CI 1.92 to 12.72, ($p = 0.0009$). However, despite an 8.95% higher proportion of patients achieving a partial response in the ER-positive cancers compared to the ER-negative cancers, this difference was not statistically significant (OR 1.50, CI 0.86 to 2.62, $p = 0.1559$).
Conclusion: There is no difference with regards to achieving a partial response to NACT in ER-positive and ER-negative breast cancers. One limitation of this study is that no multivariate analysis was conducted. Nonetheless, a partial response is similarly effective at enabling breast conservation. Despite this, patients may still opt for a mastectomy over a lumpectomy.

P1.10

The impact of lymphovascular invasion as a prognostic indicator in the TransATAC study

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Purpose: Lymphovascular invasion (LVI) is a known prognostic factor in patients with breast cancer. However, LVI is not included in the international TNM staging system. The current study aimed to investigate the impact of LVI for prognosis in ER+ postmenopausal women in the TransATAC trial, the translational substudy of the Arimidex, Tamoxifen, Alone or in Combination trial (ATAC). Here, we focus on determining if LVI adds independent prognostic information to the Clinical Treatment Score (CTS), based on tumour size, nodal status, grade, age and treatment.
Methods: LVI was recorded as present or absent in a set of 1209 patients with ER+ primary breast cancer treated with anastrozole or tamoxifen for 5 years and included in the TransATAC cohort. We used Cox regression models to determine the prognostic value of LVI and Kaplan–Meier graphs to compare 10-year distant recurrence (DR).
Results: Patients with no LVI present had better outcome ($n = 178$, DR 13.8%) against those with LVI present ($n = 1031$, DR 24.2%, HR 1.88, 95% CI (1.33–2.64); $p = < 0.0001$, LR-$\chi^2 = 11.61$; $p = < 0.007$) over a 10-year period. LVI presence added significant prognostic information beyond IHC4 in years 0–10 (LR-$\chi^2 = 8.9$) but added little to the CTS (LR-$\chi^2 = 0.8$). For years 5–10, LVI again

added significant prognostic information beyond IHC4 (LR-$\chi^2 = 3$) while LVI added no significant information to CTS (LR-$\chi^2 = 0.9$). LVI was found to be more prevalent within the HER2-enriched subtype (34%) than Basal-Like (25%), Luminal A (12%) or Luminal B (17%). LVI presence in the HER2-negative and Node-negative subtype was found to be more favourable (DR 6.9%, HR 0.77, 95% CI (0.31–1.93); $p = 0.58$) than LVI absence within this subtype (DR 8.7%).

Conclusion: We have demonstrated in the TransATAC study that the presence of LVI leads to poorer patient outcomes than in those patients without LVI. Furthermore, LVI presence had a significant impact to the IHC4 score.

P1.11

Intra-operative detection of tumor at lumpectomy surface during breast conserving surgery by multimodal spectral histopathology

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We present multimodal spectral histopathology (MSH), a technique capable of intra-operative detection of residual tumor on the surface of breast excision specimens. MSH employs rapid tissue imaging by confocal auto-fluorescence to guide molecular analysis by Raman spectroscopy. A total of 214 breast tissue samples were used to train (91 samples from 65 patients) and independently test (123 samples from 108 patients) automated data processing and diagnosis algorithms. The independent test yielded 91% sensitivity and 82% specificity for detection of breast carcinoma on specimens' surface. Notably, MSH was able to detect residual tumors at the excision margin of all ten positive lumpectomy specimens, including invasive carcinoma and ductal carcinoma in situ (DCIS) with tumors as small as 1.3×1.3 mm^2 that can often be missed during surgery. While our laboratory-grade instrument allowed tissue areas as large as 5×7.5 cm^2 to be analyzed within 25 min, future optimization and automation can further increase the speed of MSH. This would provide an objective tool for intra-operative assessment of breast conserving surgery margins, guiding surgical intervention to maintain cosmesis and reduce unnecessary second operations.

P1.12

Automated Ki-67 assessment: From invasive tumor component detection to Ki-67 quantification in hot spots

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Objective: To investigate how the level of automation for Ki-67 assessment using digital image analysis (DIA) can be increased using a new DIA workflow, and to compare the new DIA workflow to a conventional DIA workflow.

Methods: A set of 84 digital images of serial whole tissue sections, one double-stained with CK7/19 and p63 and the other stained with the analytical marker Ki-67, were aligned using the VirtualDoubleStaining™ technique. The aligned images were then analyzed using two different DIA workflows both developed by Visiopharm A/S. The first DIA workflow identified tumor regions based on the CK7/19 stained slide, transferred these to the Ki-67 slide and quantitated the Ki-67 proliferation index within the identified tumor components on the whole slide. The new DIA workflow identified tumor regions and separated these into invasive and non-invasive tumor regions based on the CK7/19 and p63 staining, transferred these to the Ki-67 slide, identified a hot spot based on the invasive tumor components only, and quantitated the Ki-67 proliferation index within the hot spot. The results produced by each of the two methods were compared to one another.

Results: An R^2 of 0.77 was obtained between the two methods, and a paired t test showed that the two DIA workflows produced significantly different results from one another ($p < 0.05$). It was found that the new DIA workflow on average gives a 20% higher Ki-67 proliferation index.

Conclusion: The presented new DIA workflow increases the level of automation for Ki-67 assessment and reduces the need for manual interaction, for example, by being able to automatically discard ductal carcinoma in situ components (i.e. non-invasive tumor). As the assessment of Ki-67 in hot spots, rather than as an average of the entire tumor area, is currently being discussed and implemented into some scoring guidelines, we here present a method for conducting this type of analysis.

P1.13

ER, PR and HER2 biomarkers in UK and Irish clinical breast cancer testing: analysis of results from > 168,000 patients

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Aims: To describe and analyse > 168,000 sets of results from clinical breast biomarker testing carried-out between 2009 and 2016 in the UK and Republic of Ireland, focusing on biological relationships. To present robust confirmatory evidence on known associations and provide new data on previously undescribed or unconfirmed ones.

Background: Between Jan 2009 and Jan 2016, the UK National External Quality Assessment Scheme for Immunocytochemistry and In situ Hybridisation (UK NEQAS ICC & ISH) collected results on clinical breast cancer testing, comprehensively for human epidermal growth factor receptor-2 (HER2) and optionally for oestrogen receptor (ER) and progesterone receptor (PR), from most UK and Irish testing centres. Primary objectives were to assess and, where indicated achieve improvements in testing quality. The size and scope of the dataset is unparalleled in the literature and represent a significant reference resource for the breast cancer community.

Methods: UK NEQAS ICC&ISH created and curated an online data-entry system allowing centres to systematically collect their own HER2, ER and PR results from clinical testing; for HER2, immunohistochemical (IHC) and in situ hybridisation (ISH) data were

collected; for ER and PR, IHC results could be entered according to local practice format. Tools and guidance were provided enabling centres to analyse their data for local audit and quality assurance. Clinicopathologic data were also collected, including patient age at diagnosis, histological tumor type, tumor grade, site/stage (primary, recurrence, metastasis) and sample type (core, excision).

Results: Data are present on 168,793 patients. 173 centres contributed $\geq$ 100 entries (96% of total). Median age was 62 years (IQR 51–72). Tumor type was stated in 42: 76% were invasive ductal, and 13% invasive lobular carcinoma. Grade was stated in 56: 15% were Grade 1, 55% Grade 2 and 30% Grade 3. Site/stage was stated in 56: 92% were primary, 2% recurrent and 6% metastatic. Sample type was stated in 70: 75% were cores and 25%, excision.

Receptor statuses were available as follows: HER2 (100%): 87% negative, 13% positive; ER (45%): 15% negative, 85% positive; PR (31%): 29% negative, 71% positive.

HER2 data were available as follows: category by IHC 91%; amplification status by ISH 15%; *HER2* gene/chromosome 17 (CEP17) ratio: 15% (86% of which were IHC 2+); *HER2* gene copy number 7%; CEP17 copy number 7%.

HER2-positive rate was 24% in ER-negative and 11% in ER-positive cases. HER2-positive status was negatively associated with increasing ER positivity (rho $-$ 0.22, $p < 0.001$). 71% of HER2-positive cases were ER-positive and 29% were ER-negative. The HER2 2+ rate was 14% in ER-negative and 21% in ER-positive cases. Considering only IHC 2+ cases, median *HER2* copy number was 5.4 in ER-negative and 4.4 in ER-positive disease.

Comprehensive description of significant associations for all parameters will be presented.

Conclusion: The unique size and scope of this dataset has allowed confirmation of known associations for HER2, ER and PR with clinicopathologic and biological characteristics in breast cancer to very high confidence levels, and has uncovered previously undescribed relationships in both ER-positive and ER-negative disease.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P1.14

Development of an automated image analysis application for Ki67 staining

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Background: Where histopathological assessment is an entry criterion in multi-centre clinical trials, standardised, reproducible methods are crucial. Such methods do exist for the assessment of Ki67 [1], but these are not consistently applied. Using recent innovations in semi-automated image analysis (IA), we carried out IA on a well-validated set of breast cancer core biopsies. Results were compared to manual scores with the aim of implementing IA for reliably assessing Ki67.

Methods: Needle core biopsies came from a selected group of patients ($n = 198$) who entered the POETIC (CRUK/07/015) clinical trial. Biopsies were collected at diagnosis and surgery. Expression of Ki67 was centrally assessed by manual counting. A HistoQuest (TissueGnostics, Austria) algorithm was developed to assess Ki67 in the same sample set. The algorithm was applied to a training-set of ten biopsy pairs, followed by a validation set of 50 pairs and 98 surgical biopsies. Agreement between manual and HistoQuest scores was assessed using Pearson's correlation coefficient. Cut-points were used to categorise Ki67 expression as low ($< 8\%$), intermediate ($\geq 8\%$ but $< 20\%$) or high ($\geq 20\%$) and categorical agreement between manual and automated results assessed.

Results: Correlation was $r = 0.86$ in the validation set and $r = 0.87$ in the surgical set (both $p < 0.0001$). Mean Ki67 score by manual assessment was $x = 10.01$ (SD = 10.25) and by IA assessment $x = 10.45$ (SD = 11.58). The overall concordance for manual versus automated scores was 72/96 (75%) in surgical set; low-risk scores agreed in 45/50 (90%), intermediate 15/31 (48.4%) and high 12/15 (80%). Weak or non-specific staining contribute to reduced concordance.

Conclusion: We show feasibility of IA use for assessment of Ki67, avoiding the limitations of manual methods.

Reference

1. Leung, S.C.Y. et al. (2016). Analytical validation of a standardised scoring protocol for Ki67: Phase 3 of an international multicenter collaboration. NPJ Breast Cancer; 2: 16014.

We are grateful to the POETIC trialists for access to Ki67 images.

P1.15

Tumour-infiltrating lymphocytes is an independent prognostic factor in breast ductal carcinoma in situ (DCIS)

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Background: Immune microenvironment plays a vital role in tumour progression. Tumour-infiltrating lymphocytes (TILs) provide prognostic significance in invasive breast cancer (IBC). Guidelines for TILs assessment in IBC, but not ductal carcinoma in situ (DCIS), have been published. This study aims to evaluate various methods for TILs assessment in DCIS and their prognostic significance.

Methods: H&E sections from two DCIS cohorts: a training set ($n = 119$) and a validation set ($n = 534$) were assessed for TILs. Seven different assessment methods were used in the training set to identify the optimal in terms of reproducibility and association with tumour recurrence which was subsequently validated.

Results: TILs touching the ducts' basement membrane or away from it by one lymphocyte cell thickness showed significant association with DCIS recurrence and highest concordance rate (inter-cluster correlation coefficient = 0.95). Assessment of TILs at wider distances from DCIS (0.2, 0.5, and 1 mm as well as stromal TILs percentage) showed prognostic significance but with lower concordance rate and were practically challenging. Assessment of TILs hotspots and lymphoid follicles did not show statistical significance with recurrence. Dense TILs were associated with younger age, symptomatic presentation, larger size, higher nuclear grade, comedo necrosis and oestrogen receptor negativity as well as shorter recurrence-free interval (RFI; $p = 0.002$). In multivariate survival analysis, dense TILs were independent predictor of shorter RFI ($p = 0.001$) in patients treated with breast conserving surgery.

Conclusion: TILs are independent prognostic variables in DCIS. Touching TILs provides a reproducible method for its assessment that can be used to guide management.

P1.16

Prolyl-4-hydroxylase α subunit 2 is associated with poor outcome in breast ductal carcinoma in situ (DCIS)

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Abstract

Background: Extracellular matrix (ECM) plays a crucial role in tumour behaviour. Prolyl-4-hydroxylase-A2 (P4HA2) is a key enzyme in collagen biosynthesis and ECM remodelling. High P4HA2 expression is associated with poor outcome in invasive breast carcinoma (IBC); however, its role in preinvasive tumours is unclear. Here, we aimed to evaluate P4HA2 expression in breast ductal carcinoma in situ (DCIS) and assess its prognostic value.

Methods: Tissue microarray (TMA) was constructed from a large DCIS cohort ($n = 750$ of pure DCIS and $n = 239$ for DCIS associated with IBC (DCIS/IBC)). TMA sections were stained for P4HA2 immunohistochemically following antibody specificity and optimisation testing and scored in tumour cells and surrounding fibroblasts. Correlation between protein expression and clinicopathological parameters and recurrence-free interval (RFI) was performed in pure DCIS cohort. Differential protein expression levels between pure DCIS and DCIS/IBC were analysed.

Results: In pure DCIS, high P4HA2 expression was detected in 51 and 25% of cases within the tumour cells and surrounding fibroblasts, respectively. High expression was associated with higher nuclear grade, comedo necrosis, oestrogen receptor and progesterone receptor negativity. Only high expression in tumour cells was associated with shorter RFI ($p = 0.001$) and this was independent of other prognostic variables. DCIS associated with IBC showed higher P4HA2 expression than pure DCIS either within tumour cells ($p = 0.003$) or fibroblasts ($p = 0.00001$). Within the DCIS/IBC mixed cases; P4HA2 stromal expression was higher in invasive component than in DCIS component ($p = 2.3 \times 10^{-8}$).

Conclusion: P4HA2 is an independent prognostic factor for DCIS recurrence and has a potential role in disease progression to IBC through its role in ECM remodelling.

P1.17

Molecular composition of breast cancer datasets must be considered for robust integrated re-analysis

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Gene expression profiling of tumors has been widely performed. Datasets' size has often been driven by sample availability and cost, leading to underpowered results. Publicly available datasets are increasingly being mined to generate new hypotheses or validate findings. Direct integration of datasets presents opportunities for meta-analysis and has the potential to improve statistical power and the generalisability of results. Between dataset 'batch-effects' due to processing time, protocols and platforms have been described and can largely be minimized. Here we clearly demonstrate that gene expression profiles can be significantly distorted if datasets with distinct molecular compositions are integrated using conventional batch correction strategies, leading to unreliable results.

Composition-adjusted batch correction methods maintain biological variation, transcriptional authenticity, and improve concordance between subtype or prognostic group assignments from single and integrated datasets (improving κ values from 60 to 97%) regardless of gene expression platform, pre-processing, or batch correction methods. This study is the first comprehensive assessment of how differences in the molecular composition of tumor datasets can affect the reliability of combining studies to increase the statistical power for robust meta-analysis. Composition-adjusted batch correction improves concordance and transcriptional fidelity.

P1.18

Multi-parameter FACS sorting for identification of subclonal endocrine resistance of ER+ breast cancer

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Background: Neoadjuvant treatment of breast cancer with aromatase inhibitors (AIs) leads to marked reduction in proliferation in most tumours. However proliferating cells remain in most treated cancers and might be responsible for the outgrowth of resistant clones. In this study, we have investigated flow cytometric cell sorting (FACS) to identify and isolate those persistently proliferating tumour cells for future genetic analysis.

Methods: Different digestion protocols were tested to obtain single-cell suspensions from 50 µ sections of FFPE breast cancer excisions. The best results were acquired using a collagenase/dispase method [1], combining heat pretreatment in sodium citrate followed by enzymatic dissociation. Cells were simultaneously stained for cytokeratin (AlexaFluor488), vimentin (AlexaFluor647), and DNA (DAPI) to distinguish between epithelial and stromal cells. Epithelial cells were further subdivided according to cell cycle into G0/G1 (mainly non-proliferating) and S/G2M (proliferating) populations using DNA histogram gating. Cells from different subpopulations were sorted and DNA subsequently extracted. The method was applied to untreated and AI-treated breast tumours.

Results: In all samples, distinct cytokeratin-positive and vimentin-positive populations were identified, which enabled sorting of different cell subpopulations. To isolate around 50 ng of total DNA (suitable for standard NGS), a minimum of 30,000 stromal cells or diploid tumour G0/G1 cells were required; more DNA was obtained from the same number of G0/G1 aneuploid tumour cells and replicating cells (S/G2M population). Over 50 ng DNA was obtained from each fraction from 70% of excision biopsies after neoadjuvant treatment, demonstrating the viability means to genetically characterise proliferative and non-proliferative tumour cell populations. Ongoing analysis involves sequencing of the extracted DNA to assess alterations in genes driving proliferation in different tumour subpopulations.

Conclusion: This methodology appears suitable for separating proliferating (potentially AI-resistant) tumour cell subpopulations for further genetic analysis.

Reference

1. Corver, W.E. et al. (2005). J. Pathol 206(2):233-41

P1.19

Development and validation of computation algorithms to compute OncotypeDX Recurrence Score and EndoPredict prognostic score from NanoString expression profiles

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To predict the likelihood of distant recurrence in patients with oestrogen-receptor positive (ER+) breast cancer, multi-gene prognostic signatures are widely used including the Oncotype DX recurrence score (RS) and EndoPredict (EP) score, both based on commercial real-time reverse transcriptase PCR (RT-PCR) in formalin-fixed, paraffin-embedded (FFPE) tissue. Expression profiling by NanoString nCounter provides a robust alternative to assay multiple genes in a single run. To accurately calculate RS and EP scores from NanoString data using the commercial algorithms, we developed a robust computation algorithm based on conversion factors derived from individual gene expression of matched NanoString and commercial analyses.

Expression of genes in the RS and EP signatures was measured by NanoString in RNA tumour extracts from 60 postmenopausal women with early-stage, ER+ breast cancer from the translational Arimidex, Tamoxifen, Alone or in Combination study (TransATAC) where these commercial signatures had been assessed by RT-PCR (Dowsett et al. JCO 2010; Buus et al. JNCI 2016). Multiple random samplings into training and validation sets allowed cross-validation and repeated evaluation of the conversion factors. Assuming linear associations between the gene expression measured by NanoString and commercial RT-PCR, linear regression models were fitted to obtain conversion factors for each gene. NanoString expression values were adjusted by the averaged factors, and RS and EP scores were calculated using the well-established algorithms.

The concordance between the commercial and corrected scaled risk scores was high (concordance correlation coefficient for RS: r(c) = 0.89, 95% CI 0.83–0.93; for EP: r(c) = 0.97, 95% CI 0.95–0.98). We found strong agreement in risk stratification by prognostic scores based on the derived NanoString values and the commercial RT-PCR values ($\kappa = 0.86$, $p < 0.0001$ for RS; $\kappa = 0.83$, $p < 0.0001$ for EP). These findings confirm the validity of our algorithms to create RS and EP prognostic scores using NanoString expression data providing a cost-effective approach to assessing these scores in research studies.

P1.20

Glutamine Transporter SLC7A5 is key therapeutic target in Luminal B breast cancer driven by MYC

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Background: Breast cancer (BC) is a heterogeneous disease characterised by variant biology and patient outcome. The amino acid transporter, SLC7A5, plays a role in BC although its impact on patient outcome in different BC subtypes remains to be validated. This study aimed to determine whether SLC7A5 is over-expressed to support BC proliferation, particularly in the highly proliferative more aggressive subtypes.
Methods: SLC7A5 was assessed at the genomic, using METABRIC data; $n = 1980$, and proteomic, using immunohistochemistry and TMA ($n = 1110$ training and $n = 1554$ validation sets) levels in well-characterised primary BC cohorts. SLC7A5 expression was correlated with clinicopathological and biological parameters, molecular subtypes and patient outcome.
Results: SLC7A5 mRNA and protein expression were strongly correlated with larger tumour size, higher grade, where high expression was observed in triple-negative (TN), HER2+ and luminal B subtypes. SLC7A5 mRNA and protein expression was significantly associated with c-MYC expression but specifically only in Luminal B tumours ($p = 0.001$).
High expression of SLC7A5 mRNA and protein was associated with poor patient outcome ($p < 0.001$) but only in the highly proliferative ER+ subclass ($p = 0.007$) and HER2 + BC, irrespective of its ER status ($p = 0.03$).
Conclusions: SLC7A5 appears to play a role in the aggressive highly proliferative ER+ subtype driven by MYC and could act as a potential therapeutic target. Functional assessment is necessary to reveal the specific role played by this transporter in this highly proliferative subclass and HER2+ BC.

P1.21

Development and clinical value of RNA-sequencing-based classifiers for prediction of the five conventional breast cancer biomarkers: a report from the population-based multicenter SCAN-B study

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Background: In early breast cancer, five histopathological biomarkers are part of current clinical routines and used for determining prognosis and treatment: estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (ERBB2/HER2), Ki67, and Nottingham histological grade (NHG). We aimed to develop classifiers for these biomarkers based on tumor mRNA-sequencing (RNA-seq), compare classification performance to conventional histopathology, and test whether RNA-seq-based predictors could add value for patient risk-stratification.
Patients and methods: In total, 3678 breast tumors were studied. For 405 breast tumors in the training cohort, a comprehensive histopathological biomarker evaluation was performed by three pathology readings to estimate interpathologist variability on the original diagnostic slides as well as on repeat immunostains for this study, and the consensus biomarker status for all five conventional biomarkers was determined. Whole transcriptome gene expression profiling was performed by RNA-sequencing on the Illumina platform. Using RNA-Seq-derived tumor gene expression data as input, single-gene classifiers (SGC) and multi-gene classifiers (MGC) were

trained on the consensus pathology biomarker labels. The trained classifiers were tested on an independent prospective population-based series of 3273 primary breast cancer cases from the multicenter SCAN-B study with median 52-month follow-up (ClinicalTrials.gov identifier NCT02306096), and classifications were evaluated by agreement statistics and by Kaplan–Meier and Cox regression survival analyses.

Results: For the histopathological evaluation, pathologist evaluation concordance was high for ER, PgR, and HER2 (average kappa values of 0.920, 0.891, and 0.899, respectively), but moderate for Ki67 and NHG (0.734 and 0.581). Classification concordance between RNA-seq classifiers and histopathology for the independent 3273-cohort was similar to that within histopathology assessments, with SGCs slightly outperforming MGCs. Importantly, patients with discordant results, classified as hormone-responsive (HoR+) by histopathology but non-hormone-responsive by MGC, presented with significantly inferior overall-survival compared to patients with concordant results. These results extended to patients with no adjuvant systemic therapy (hazard ratio, HR, 3.19; 95% confidence interval, CI 1.19–8.57), and endocrine therapy alone (HR 2.64; 95% CI 1.55–4.51). For HoR+ - cases receiving endocrine therapy alone, the MGC HoR classifier remained significant after multivariable adjustment (HR 2.45; 95% CI 1.39–4.34).

Conclusions: RNA-seq-based classifiers for the five key early breast cancer biomarkers were generally equivalent to conventional histopathology with regards to classification error rate. However, our RNA-seq classifiers provided added clinical value in particular for cases determined by histopathology to be hormone-responsive but by RNA-seq to be hormone-insensitive and have a significantly poorer outcome when treated with endocrine therapy alone.

P1.22

Development and validation of a FISH method to analyse *ESR1* in FFPE breast cancer biopsies

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Background: Mutations in *ESR1* have been associated with acquired resistance to aromatase inhibitor (AI) therapy in breast cancer. The prevalence and clinical significance of *ESR1* amplification in breast cancer has not been determined to date with different studies showing conflicting data. In this study, we developed and validated a FISH method in FFPE sections and further investigated the prevalence of *ESR1* amplification in the context of AI treatment resistance.

Methods: A commercially available ESR1/CEN6 dual colour probe was used and an RNase A pre-treatment step added to eliminate false positives. FISH scoring was performed manually by counting the number of *ESR1* and *CEN6* signals in the nucleus of at least 20 representative cells. Amplification and gains were defined as an *ESR1*/*CEN6* ratio of ≥ 2.0 and ≥ 1.5, respectively.

Results: Our method was validated in a small set of matched primary and metastatic breast cancer biopsies ($n = 3$ pairs) from the ABC-Bio study where *ESR1* amplification had previously been identified by NGS. The *ESR1*/*CEN6* ratios obtained in the metastasis tumours were 4.4, 2.3 and 3.2, confirming the amplification status and the validity of the method.

The clinical significance of *ESR1* amplification in the context of AI treatment resistance was investigated by performing *ESR1* FISH in a set of matched primary and recurrent biopsies ($n = 43$ pairs) from patients who received AI treatment. No *ESR1* amplified tumours were identified but gains were observed in five out of 43 recurrent biopsies (11.6%) and in one out of 43 primaries (2.3%). Samples with gains showed a high heterogeneity of *ESR1* signals both between individual cells and between tumour areas.

Conclusion: We have developed and validated a method for *ESR1* analysis by FISH. Amplification of *ESR1* is infrequently found in AI-resistant disease but low-level gains are more frequent. Further work is needed to determine their clinical significance.

P1.23

Do prognostic gene signatures for ER + breast cancer change during the menstrual cycle?

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Background: Differences occur in the expression of oestrogen-regulated genes and proliferation-associated genes in ER + breast tumours in premenopausal women during the menstrual cycle. Some of these genes feature in widely used multigene prognostic signatures but the impact of their change on the overall prognostic score is unknown.

Aims: To investigate if prognostic scores from Oncotype DX Recurrence Score (RS), EndoPredict (EP), Prosigna (PAM50) and Breast Cancer Index (BCI) vary during the menstrual cycle.

Methods: RNA was extracted from paired FFPE samples ($n = 25$) taken from the same ER+ tumour in different windows of the menstrual cycle. Menstrual cycle windows were prospectively defined as either W1 (early and very late cycle; days 1–6 and 27–35) or W2 (mid- to late cycle; days 7–26). Gene expression was measured using the NanoString nCounter system and prognostic signature scores calculated after applying validated conversion factors derived from 60 samples from the TransATAC study.

Results: Mean ($\pm$ SEM) scores were not significantly different between W1 and W2 for RS (29.0 ± 3.5 vs. 29.6 ± 3.6; mean difference $= -0.56 \pm 1.68$) and EP (6.9 ± 0.52 vs. 7.1 ± 0.54; mean difference $= -0.17 \pm 0.34$) and the two measurements showed high correlation (RS, $r = 0.91$; EP, $r = 0.83$). Nonetheless, for RS, EP and EPclin, 7/25, 2/25 and 3/25 of tumours, respectively, were assigned to a different risk category in W2. Within the RS, the ER module score was higher in W2 (+ 15.7%; $p = 0.048$), the invasion module score was lower (− 16.1%; $p = 0.028$) and there was a trend for higher proliferation module score (+ 8.1%; $p = 0.068$). Two individual genes showed a significant difference between the windows: PGR (+ 71.6%; $p = 0.0057$) and MMP11 (− 22.1%, $p = 0.034$). Data for PAM50 and BCI will be shown.

Conclusions: Significant changes in gene and gene module scores occurred during the menstrual cycle but these did not affect RS or EP in a systematic fashion.

P1.24

Genomic characterization of contemporary population-based metastatic breast cancer in Southern Sweden through the SCAN-B-rec network

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Background: Despite great improvements in breast cancer treatment, many women with primary disease still develop metastases (mBC). Due to the genomic evolution during tumor progression and treatment pressure, improved molecular characterization beyond current practice and in addition to primary tumor characterization is of increasing importance to guide the use of future targeted therapeutics of mBC
Materials and methods: A collaborative network (SCAN-B-rec) of clinicians and pre-clinical scientists is being established within the south Swedish healthcare region, building on experiences and networks from the SCAN-B (South Swedish Cancerome Analysis Network—Breast) project. mBC patients will be prospectively recruited based on informed consent specific for SCAN-B-rec. Formalin-fixed paraffin-embedded (FFPE) tissues are collected from diagnostic mBC tissue with additional SCAN-B-rec specific fresh tissue biopsies from selected patients. Mutations and copy number alterations in key breast cancer-related genes will be analyzed continuously by NGS.
Results: Based on experience from clinical NGS screening in lung cancer and malignant melanoma, a central laboratory at the Department of Oncology and Pathology, Lund University, has been established. A pilot study of 23 mBCs (FFPE) revealed that 60% meet stringent DNA quality parameters for targeted NGS, whereas another 27% meet minimum requirements. Sequencing revealed both cases with matching genomic profiles compared to primary lesions, and cases with occurrence of new driver alterations after adjuvant treatment.
Implications: Improved genomic characterization and understanding of tumor evolution in different breast cancer subtypes and during different adjuvant treatments will aid the introduction of targeted therapeutics for mBC patients along treatment regimes.

P1.25

Clinical impact of molecular subtype and risk of recurrence score in early breast cancer patients with long-term follow-up

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Background: The aim of this study was to investigate the prognostic value of the PAM50 intrinsic subtypes and risk of recurrence (ROR) score in patients with early breast cancer and long-term follow-up. A special focus was placed on the hormone receptor-positive/HER2-negative (HR+/HER2) pN0 patients of whom the majority did not receive chemotherapy due to the prevailing guidelines during study inclusion.
Methods: Patients with early breast cancer ($n = 653$) enrolled in the observational Oslo1-study (1995–1998). Primary tumors were analyzed using the Prosigna PAM50 gene signature to determine the prognostic value of the intrinsic subtypes and ROR score in comparison with pathologic characteristics. The primary endpoints were distant disease-free survival (DDFS) and breast cancer-specific survival (BCSS).
Results: Of 653 tumors, 52.2% were classified as luminal A, 26.5% as luminal B, 10.6% as HER2-enriched, and 10.7% as basal-like. Among the HR+/HER2− patients ($n = 476$), 37.8% were categorized as low-risk by ROR score, 22.7% as intermediate-risk, and 39.5% as high-risk. Median follow-up for BCSS and DDFS was 16.6 years and 7.1, respectively. Multivariate analysis showed that the intrinsic subtypes (all patients) and the ROR risk-classification (HR+/HER2− patients) yielded strong prognostic information. Among the HR+/HER2− pN0 patients with no adjuvant treatment ($n = 231$), 53.7% of patients had a low ROR and their prognosis at 15 years was excellent (15 years BCSS 96.3%). The survival for the intermediate-risk group was reduced compared to the low-risk group ($p = 0.005$). Furthermore, 55% of patients who according to PREDICT would be considered chemotherapy candidates was ROR low-risk (33%) or luminal A ROR intermediate-risk (22%).
Conclusions: The PAM50 intrinsic subtype classification and ROR score improve the classification of breast cancer patients into prognostic groups, allowing for a more precise identification of future recurrence risk and provide an improved basis for adjuvant treatment decisions.

P1.26

Characterisation of HER2 status in DCIS using immunohistochemistry (IHC) and chromogenic in situ *h*ybridisation (CISH)

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Background: Previous studies have reported high percent (up to 60%) of ductal carcinoma in situ (DCIS), being HER2+. However, the frequency of HER2+ in IBC is lower and ranges from 10 to 20%. The aim of this study is to characterise HER2 status in DCIS and assess its prognostic value.
Methods: Tissue microarrays (TMAs) were constructed from a large and annotated series of DCIS comprising pure ($n = 777$) and mixed (DCIS associated with IBC; $n = 239$) DCIS. HER2 status was evaluated at the protein level using immunohistochemistry (IHC) and the gene levels using CISH according to the published HER2 guidelines recommendation for IBC.
Results: Six hundred and twenty-five pure DCIS cases were valid for IHC assessment. HER2 negative (0/1+) DCIS tumours represented 76.3% (477/625) of the whole cohort, whereas HER2 2+ (equivocal) was present in 15.4% (96/625) and HER2 3+ status was identified in 8.3% (52/625). Six hundred and seventy-seven pure DCIS cases were available for CISH scoring. CISH did not detect HER2 gene amplification in 79.7% of DCIS cases (539/677). In IHC equivocal cases,

HER2 amplification (tumours showing mean HER2 gene copy number ≥ 6 signals per nucleus) was confirmed by CISH in 74% (71/96). CISH confirmed high HER2 copy number in all IHC 3+ cases (52/52). The final HER2 + status of pure DCIS, confirmed by CISH, represented 20.4% of the total cohort (138/677).
In mixed DCIS cases, HER2 amplification of the DCIS component was detected in 14.9% with amplification of invasive component represented only 12.6%.

HER2+ DCIS was associated with high nuclear grade ($p = 6.3 \times 10^{-13}$), comedo type DCIS ($p = 0.005$), larger tumour size ($p = 2 \times 10^{-6}$) and negative hormone receptor status ($p = 3.4 \times 10^{-21}$).
Conclusions: Our results indicate the frequency of HER2 positivity in DCIS is comparable to IBC and that HER2+ DCIS is associated with features of poor prognosis. Similar to IBC, the majority of HER2 overexpression in DCIS is driven by gene amplification.

P1.27

Stromal tumour lymphocyte infiltration as a prognostic biomarker in triple-negative breast cancer

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Background: Stromal tumour-infiltrating lymphocytes (sTILs) are emerging as a potential prognostic and predictive biomarker in triple-negative breast cancers (TNBC). In this study, we tested the prognostic role of sTILs in a retrospective series of TNBCs patients.
Methods: sTILs were evaluated according to the TILs International Working Group Guidelines in full face sections of 303 TNBCs. Lymphocyte-high (LHBC) and Lymphocyte-predominant breast cancer (LPBC) were defined as sTILs $\geq 25\%$ and $\geq 50\%$, respectively. The association between sTILs and clinic-pathological variables and between sTILs and outcome was determined with sTILs expressed in 10% increments, as LHBC and as LPBC.
Results: The median sTILs count in the TNBC series was 15% (range 0–90%; mean 22%). In the entire cohort, 30% of cases were categorised as LHBC and 16% as LPBC LHBC was significantly associated with histological type, poorly differentiated tumours, and high nuclear grade ($p \leq 0.001$, $p = 0.047$, $p = 0.029$). On multivariable analysis, sTILs expressed as a binary variable using a 25% threshold (LHBC) was an independent predictor of disease-free-survival (DFS) (HR 0.44, 95% CI 0.25–0.79, $p = 0.007$) and overall-survival (OS) (HR 0.56, 95% CI 0.32–0.99, $p = 0.047$) when adjusted for age, tumour type, tumour grade and nodal status. The association between LPBC (sTILs $> 50\%$) and DFS or OS was not significant on multivariable analysis (HR 0.48, 95% CI 0.22–1.06, $p = 0.07$; HR 0.59, 95% CI 0.28–1.23, $p = 0.162$, respectively).
Conclusion: Our findings support a prognostic role for sTILs in TNBC. sTIL-high ($> 25\%$) was an independent predictor of survival. The lack of prognostic significance of LPBC may be due to the small numbers in this category.

P1.28

OncotypeDX Recurrence Score distributions in invasive ductal and lobular breast cancer differ: an analysis of TransATAC and clinical testing cases

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Background: Invasive lobular carcinoma (ILC) comprises approximately 15% of early breast cancer cases. Its pathology and clinical course differ from invasive ductal carcinoma (IDC), and has been widely reported that the distribution of OncotypeDX Recurrence Score (RS) is also different, being lower in ILC compared to IDC populations.
Aim

(1) To compare the proportions of patients with IDC or ILC disease in TransATAC and our clinical testing group that received low-risk versus non-low-risk RS results, stratified for clinical risk using the Nottingham Prognostic Index (NPI).
(2) To compare survival in TransATAC patients stratified by disease type and RS risk.

Materials and methods: The TransATAC set comprised 806 (84%) IDC cases and 151 (16%) ILC cases. The clinical testing set was composed of tumours tested between 2012 and 2017; 178 (81%) were IDC and 43 (19%) ILC. All cases were ER-positive, HER2-negative. Cases were stratified unto NPI and RS risk groups using standard cut-points. Kaplan–Meier survival curves and hazard ratios were generated for the TransATAC data.
Results: In TransATAC IDC cases, median RS was 16 (IQR 11–24), for ILC it was 15 (IQR 11–20). In clinical IDC cases, median RS was 17 (IQR 12–23), for ILC it was 13 (IQR 10–18). In the NPI-moderate (NPI > 3.40 but ≤ 5.40) TransATAC and clinical cohorts, proportions of cases within tumour type defined by RS as low risk were very similar; TransATAC IDC 45% low, clinical IDC 49%; TransATAC ILC 71% low, clinical ILC 68%. In both case-series, difference in proportions of low and not-low-risk cases for each tumour type was significant (TransATAC $p = 0.0003$, clinical $p = 0.034$).
Comparing outcome data in TransATAC: for IDC, the HR (low vs intermediate) was 1.51 (0.99–2.30), for ILC it was 2.35 (1.02–5.44), suggesting that RS scores are valid when used to aid decision making on treatment options in patients with ILC.
Conclusions: RS results in ILC cases tend to be lower than in IDC but retain the same relationship with residual risk of distant recurrence.

P1.29

Use of ctDNA in the detection of emerging ESR1 mutations in endocrine therapy resistance

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ESR1 mutations are rare in primary breast cancer but have a high prevalence in advanced disease previously treated with AI, implying evolution through selective treatment pressure. To investigate acquisition/prevalence of *ESR1* mutations in metastatic breast cancer (MBC) patients, we developed high-sensitivity multiplex dPCR assays for *ESR1* mutations in ctDNA and investigated the origin and clinical relevance of *ESR1* mutations in advanced breast cancer.
In a retrospective proof-of-principle study on 171 MBC, *ESR1* mutation status in ctDNA showed high concordance with contemporaneous tumour biopsies. We found *ESR1* mutations exclusively in ER+ breast cancer patients previously exposed to AI. Patients with *ESR1* mutations had shorter PFS on subsequent AI-based therapy [HR 3.1; 95% CI 1.9–23.1; $p = 0.0041$]. The prevalence of mutations differed between patients who were first exposed to AI during the adjuvant and metastatic settings [5.8% (3 of 52) vs. 36.4% (16 of 44), respectively; $p = 0.0002$].

We further examined *ESR1* mutations occurrence in another retrospective study utilising plasma samples from the SoFEA trial (comparing exemestane with fulvestrant-containing regimens) and the PALOMA-3 trial (comparing fulvestrant plus placebo with fulvestrant plus palbociclib) in patients with prior sensitivity to AI. In SoFEA, *ESR1* mutations were found in 39.1% of patients, these patients had improved PFS after taking fulvestrant compared with exemestane ($p = 0.02$), while *ESR1* wild-type patients had a similar PFS on both treatments ($p = 0.77$). In PALOMA-3, 25.3% of patients had ESR1 mutations. Fulvestrant plus palbociclib improved PFS, compared to Fulvestrant alone, in both *ESR1* mutant and *ESR1* wild-type patients ($p = 0.002$ and 0.001, respectively).

We demonstrated that *ESR1* mutations can be identified with ctDNA analysis and predict for resistance to subsequent AI therapy by showing that *ESR1* mutations are rarely acquired during adjuvant AI but are commonly selected by therapy for metastatic disease, providing evidence of mechanisms of resistance to targeted therapy. Identification/characterising of the changes occurring during postoperative AI treatment might help to direct the choice of further endocrine-based therapy.

P1.30

The NanoString immune panel accurately classifies patients according to their level of immune cell infiltration

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The tumor microenvironment considerably influences tumor progression and clinical outcome[1]. We and others have shown that the immune response in breast adenocarcinoma and adjacent normal tissue is dependent on age and estrogen availability[2]. Furthermore, immune infiltration has been associated with disease progression, ER activity, and genomic complexity[3]. Therefore, characterizing the relationship between tumors and their microenvironment through bioinformatics approaches will increase our understanding of pathogenesis[4].

Here, we used the nCounter® System on RNA extracted from FFPE samples, to measure the expression of 760 genes related to immune system and immune response (the immune panel) in a test dataset (OslI, $n = 96$). The expression of the same 760 genes measured in FFPE was compared to that obtained from fresh frozen sample from the same tumors (OslI, $n = 96$) by Agilent array (44 K). Results were validated on different platforms in different breast cancer cohorts by Agilent array (60 K) (OslII, $n = 277$ and METABRIC, $n = 1980$) as well as RNA-seq (TCGA, $n = 981$). In all cases, three clusters of patients were identified. The three clusters were further found to reflect patients with different levels of lymphoid and myeloid infiltration. ER-negative breast cancer patients partitioned in the cluster with highest myeloid and lymphoid infiltration had a better prognosis. Finally, we used deconvolution algorithms to infer for the presence of more precise immune cell types which may associate with prognosis in ER-negative breast cancer.

In conclusion, based on the expression of 760 genes (the NanoString Immune panel), we can identify three clusters of patients with different immune cell infiltrations also associated with prognosis in ER-negative breast cancer. These three clusters may serve to understand the interrelation between tumor and immune response parameters.

[1]Quigley et al. 2015 PMID:26607741

[2]Quigley et al. 2017 et al. http://www.tandfonline.com/doi/abs/10.1080/2162402X.2017.1356142

[3]Dannenfelser R et al. 2017 PMID: 28915659

P1.31

Identification of germline mutation using a 30-gene sequencing in breast cancer patients not found to carry BRCA mutations

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Background: With the recent discovery of other breast cancer susceptibility genes (e.g., CDH1, ATM, CHEK2, PALB2, RAD50), molecular diagnosis of hereditary breast and ovarian cancers (HBOC) using multigene panels could help to identify other moderate/low penetrance genes in patients who tested negative for BRCA mutation. However, the clinical management of these cancer predisposition genes have not been clearly defined, therefore only BRCA1 and BRCA2 are routinely included in the genetic screening. In view of the differences in

the mutation spectrum across ethnicity, it is important to identify other HBOC genes to estimate the associated breast cancer risk in Chinese.
Methods: High-risk breast cancer patients who were negative for BRCA1, BRCA2, TP53, and PTEN were selected from the Hong Kong Hereditary Breast Cancer Family Registry between 2007 and 2016. In the study, 745 patients were subjected to 30-gene panel by next-generation sequencing (Color Genomics). All detected pathogenic mutations were further validated by bi-directional DNA sequencing. The sequencing data were co-analyzed by our in-house developed bioinformatics pipeline.
Results: Thirty-five pathogenic variants were identified in this series (4.7%), which correspond to 11 different cancer predisposition genes. Majority of the carriers (74.29%) had early-onset of breast cancer (age < 45), 42.86% had ≥ 2 family members with cancer, and 17.14% were triple-negative. The most common mutated genes were PALB2 (1.21%), RAD51D (0.94%), and ATM (0.67%). However, the cancer risk of RAD51D in breast cancer warrants further investigation. Moreover, over 28% of patients had a variant of unknown significance (VUS) in these genes (excluding BRCA1, BRCA2, TP53, and PTEN), which account for 183 types of VUS. Data from large-cohort studies and international consortiums will help to define the pathogenicity and clinical interpretation/management of the variants. Further analysis are now being performed in a larger cohort.
Conclusions: Our findings suggested that detection of PALB2 should be included in the genetic test panel in Chinese with breast cancer. Multigene panel testing is an efficient tool in the diagnosis of HBOC, this could help patients to understand the cancer risk and aid the development of effective treatments. An updated finding from a larger cohort will be presented at the meeting.

P1.32

Using NHS PREDICT to evaluate outcomes at 5 years in breast cancer patients under 50 and those with multi-focal disease

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Background: PREDICT (http://www.predict.nhs.uk) is a freely available web tool that enables the input of clinical data and tumour characteristics of breast cancer patients, to determine how various pharmacological treatments may affect survival. Previously, it was noted that the tool gave less accurate predictions for younger patients[1]. Additionally, it is currently unable to account for multi-focal disease. This study aimed to validate the tool using two cohorts of consenting patients who donated tissue to the Leeds Breast Cancer Now Tissue Bank; those under 50 and those of any age with multi-focal disease.
Methods: Data from women diagnosed from 2010 to 2012 were evaluated. This comprised 130 patients under 50, and 68 with multi-focal disease. Their five-year PREDICT score was compared with their survival status 5 years after diagnosis; for those with multi-focal disease a score for each individual tumour was calculated.
Results: In the under 50 cohort (mean age 44, range 25–49), the five-year mean PREDICT score was 93.5% (range 46–99%, 95% CI 0.041, SD 7.43). The actual recorded five-year survival was 83.7%: a difference of 10.2%. 74% of multi-focal patients displayed variation in their tumour scores. A 53-year-old patient displayed an 18% change for their two scores (83; 98), along with different grades, invasive sizes, and hormone receptor statuses. In contrast, a 61-year-old patient had identical PREDICT scores (96), the same pathological classification, and only 1 mm difference in the tumour sizes.
Conclusion: This study highlighted the unpredictable nature of breast cancer in women under 50 and the subsequent difficulty in predicting mortality. Additionally, it has identified that new parameters, such as multifocality, should be accounted for in the PREDICT model to improve risk stratification. It is hoped this will improve the quality of information made available to women with breast cancer and aid in more informed clinical decision-making.

Reference

1. Maishman T, et al. B J Cancer. 2015;112(6):983–991.

P1.33

A comparison of the performance of EndoPredict Clinical and NHS PREDICT in 120 patients treated for ER$^+$ Breast Cancer

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Background: Computational algorithms, such as NHS PREDICT, were developed using cancer registry data to guide decisions regarding adjuvant chemotherapy. They are limited by biases of the underlying data. Recent breakthroughs in molecular biology have aided in the development of genomic assays which provide superior clinical information. In this study, we compare the performance in risk stratification of **EndoPredict** Clinical (EPClin, a composite of clinical data, and **EndoPredict**) and PREDICT in a cohort of breast cancer patients considered potential candidates for chemotherapy by the clinicians.
Materials and Methods: One hundred and twenty patients with biopsy-proven ER+ve/HER2−ve breast cancer who underwent surgery were included. EPClin and PREDICT were determined for every tumour, and the results were compared.
Results: Using EPClin scores performed on 120 tumours, the cohort was stratified into low ($n = 60$) and high-risk ($n = 60$) groups leading to 50% reduction in total chemotherapy prescriptions. PREDICT differentiated the patients into low ($n = 45$), intermediate ($n = 33$), and high-risk groups ($n = 42$). Discordance between scores was demonstrated for 50 (41.66%) tumours, Nine (20%) out of 45 patients with low PREDICT scores had high EPClin scores and would otherwise not have received chemotherapy if the NHS PREDICT tool had been used alone. Eight (19%) out of 42 patients at high risk by PREDICT were reclassified as being at low risk by EPClin and avoided adjuvant chemotherapy. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for NHS PREDICT to predict the potential need for chemotherapy as determined by EPClin were 85, 51, 68, and 80%, respectively.
Conclusion: To our knowledge, this is the first clinical study to compare EPClin and PREDICT. The data indicate that computational algorithms such as NHS PREDICT may not accurately predict the need for chemotherapy leading to overtreatment, undertreatment, or uncertainty and anxiety in a significant proportion of patients. This underscores the importance of more personalized prognostic tools.

P1.34

Epigenetic modulators for the treatment of invasive lobular carcinoma breast cancer

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Invasive lobular carcinoma (ILC) is a breast cancer subtype comprising 10% of breast tumours. The majority of ILC (90%) are oestrogen receptor (ER)-positive and therefore candidates for endocrine therapy. Unfortunately, de novo resistant to endocrine therapies occurs in 33% of women and a further 40% will relapse on treatment. Therefore, novel therapeutic targets are required for ILC.

Deregulated transcription is a recurring theme in cancer, which can be due to epigenetic events. The bromodomain and extra-terminal domain (BET) family of proteins (BRD2, BRD3, BRD4, BRDT) function as chromatin readers that bind acetylated lysine residues on histones and regulate transcription. We performed RNA-Sequencing analysis on 61 primary ILC samples and found that high expression of BRD3 is associated with poor survival in ILC (log rank test, $p = 0.037$). Next, we tested if ILC cell lines were sensitive to BET inhibition using the small molecule inhibitor JQ1. Pathway analysis following RNA sequencing revealed that JQ1 targets the apoptotic and Wnt signalling pathways in ILC cell lines. Interestingly, JQ1 inhibited the cell growth in all ILC cell lines tested; however, apoptosis was only induced in two ILC cell lines. Furthermore, ILC cell lines which were relatively resistant to JQ1-induced apoptosis expressed both the BCL-2 and BCL-XL anti-apoptotic proteins. This led us to assess the combination of JQ1 and the BH3 mimetics, ABT-199 and ABT-263. We found the combination of JQ1 and ABT-263, but not the combination of JQ1 and ABT-199, to be synergistic and enhance apoptosis in ILC cell lines. This is in accordance with BH3 profiling of ILC cell lines which indicated that ILC cell lines are dependent on BCL-2/BCL-XL proteins for cell survival.

Future work will include determining the specific role of BRD3 in ILC and the effectiveness of the JQ1 and ABT-263 combination in vivo. Our work suggests that inhibition of BET proteins in combination with BH3 mimetics may be a rational therapeutic combination for ILC.

P1.35

The effect of surgery on the levels of sVEGFR-2 in early breast cancer

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Background: Angiogenesis plays an important role in the pathogenesis of breast cancer (BC). VEGF is a crucial positive regulator of this process. The aim of this study was to measure the levels of sVEGFR-2 in women with BC before and after surgery to see if surgery altered sVEGFR-2 levels in case the observation in BEATRICE trial of the Bevacizumab effect in patients with high sVEGFR-2 levels was a surrogate marker for surgical intervention and time trend.

Methods: Following ethical approval, we measured the plasma concentrations of sVEGFR-2 in 30 patients with a range of early-stage BC. All patients had 7 ml of blood sample collected prior to surgery, 10–14 days and 5–6 weeks after surgery. Plasma samples were analysed in duplicate on ROCHE IMPACT ISR instrument for determination of sVEGFR-2 levels.

Results: 69% of patients have an increase in levels of sVEGFR2 at the late time point compared to pre-treatment ($p < 0.0004$). 65% of them also have increased levels at 2 weeks, with statistically significantly higher levels at the late time point compared with the early time point ($p < 0.0002$). Linear regression analysis of independent time points showed no independent variables to be associated with pre-treatment levels of sVEGFR2 (variables tested: age, grade, size, T, nodes, ER, HER2, subtype, menopause status, smoking). No independent variables were found to be associated with the increase from pre-treatment levels of sVEGFR2 at the late time point. In a combined linear regression analysis, time from surgery was significantly associated with an increase in sVEGFR2 levels.

Conclusions: We showed that surgical intervention might affect the levels of sVEGFR-2. Further research is needed to understand the factors driving plasma VEGFR-2 variance and correlation with treatment effect from anti-angiogenic markers like Bevacizumab.

P1.36

Analysis of the outcome of the intermediate Oncotype DX score in relation to the chemotherapy decision at the Royal Liverpool Hospital

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Introduction: The Oncotype DX recurrence score is directly correlated with the rate of distant recurrence in early-stage breast cancer. A high- or a low-score indicates a high or a low chemotherapy benefit, respectively. However, patients at the intermediate score group are advised to further discuss chemotherapy treatment as their actual benefit is not yet clear. Our aim is to analyse the management of patients with an intermediate result and to identify the factors that guide the decision making in this category.

Methods: All patients with an Oncotype DX score requested at the Royal Liverpool University Hospital were reviewed over a 1-year period. Patients with an intermediate score were further analysed according to their age, comorbidities, performance status, Oncotype DX score value, Nottingham prognostic index (NPI) and decision to pursue chemotherapy. The statistical analysis was performed using the SPSS 21 tool.

Results: Data were collected from August 2016 to August 2017. In total, 53 patients had an Oncotype DX assay. Of these, 49% had low, 34% intermediate, and 17% high Oncotype DX score. The mean Oncotype DX score for the intermediate group was 22.5, and 22.2% of these patients went on to have chemotherapy.

Conclusions: Oncotype DX is a validated tool to guide chemotherapy options in early-stage breast cancer for those of a high and a low score. It can lead to the omission of chemotherapy for patients that would otherwise have had that discussion. However, for patients with an intermediate Oncotype DX score, more studies are required to evaluate the factors that influence decision making and long-term survival outcomes.

P1.37

AIB1 is a new putative prognostic biomarker in the luminal A and B-like (HER2-negative) classification of invasive lobular carcinoma

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Background: Estrogen receptor (ER)-positive HER2-negative breast cancer comprises 75–80% of all breast cancer. This fraction is even higher (> 90%) in invasive lobular carcinoma (ILC). According to the St Gallen surrogate definitions of the intrinsic subtypes, Ki67 and progesterone receptor (PgR) are used to classify these tumors as luminal A- and luminal B-like (HER2-negative). These guidelines are based on information derived from patient materials with mixed histological types, where the vast majority of the patients have invasive ductal carcinoma. The 'luminal-like classification' together with histological grade, tumor size, and lymph node status is widely used in the clinic for prognostication. The aim of the present study was to investigate if the same markers are applicable for ILC, and furthermore, if additional biomarkers involved in the endocrine signaling system, e.g., amplified in breast cancer 1 (AIB1) and the putative G protein-coupled estrogen receptor (GPER), might provide complementary prognostic information.

Patients: Two hundred and thirty-three ($n = 233$) well-characterized patients with primary ILC, diagnosed between 1980 and 1991 were included. Forty-two percent of the patients received adjuvant endocrine treatment and 2% received adjuvant chemotherapy. All biomarkers were analyzed immunohistochemically on tissue microarray, whereas histological grade was evaluated on whole sections according to Elston and Ellis (NHG). The primary endpoint was breast cancer mortality (BCM).

Results: In univariable analyses with 10-year follow-up, Ki67 (high vs. low), NHG (3 vs. 1 + 2), and AIB1 (high vs. low) were significantly associated with BCM (Hazard Ratio 4.7, 95% CI 2.1–10.4, $p < 0.001$; HR 3.1, 95% CI 1.5–6.4, $p = 0.003$; HR 3.2, 95% CI 1.4–7.2, $p = 0.005$, respectively), whereas PgR (< 1% vs ≥ 1%) and GPR30 (linear 0-4) were not ($p = 0.25$; $p = 0.31$, respectively). Essentially the same effect was seen after multivariable adjustment for lymph node status (+ vs. −), tumor size (> 20 vs. ≤ 20 mm), adjuvant treatment, and age (continuous). Subgrouping the tumors into luminal A- and B-like (HER2-negative) according to St Gallen surrogate definitions did not show significant prognostic differences between the two groups ($p = 0.12$). Patients with ≤ 20 mm, lymph node-negative breast cancer and favorable tumor characteristics (low Ki67, NHG 1 + 2, and low AIB1) had a 10-year BCM of 4.2% (95% CI 1.4–12%). This group constituted 34% of the patients included in the present study.

Conclusions: In contrast to other previous studies, where breast cancers of mixed histological types were included, PgR was not significantly associated to prognosis in the ER-positive HER2-negative subgroup in the present study, consisting only of ILC. The prognostic role of PgR and the clinical usefulness of the luminal A and B-like (HER2-negative) classification (using only Ki67 and PgR) in ILC are still to be further investigated. The prognostic importance of Ki67 and NHG in this subgroup was, however, confirmed also in ILC, and AIB1 might be a new putative prognostic factor. By combining Ki67, NHG, and AIB1, together with lymph node status and tumor size, a group of patients with an excellent prognosis could be identified.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P1.38

Putting multigene signatures to the test: prognostic assessment in population-based contemporary clinical breast cancer

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Background: Gene expression signatures hold promise for a molecularly driven division of primary breast cancer with clinical implications. A gap still remains in the application/validation of such signatures in actual clinical treatment groups from unselected, population-based, primary breast cancer receiving current standard of care therapy. We analyzed classification proportions and overall-survival (OS) of 14 reported gene expression phenotypes (GEPs) and risk predictors (RPs) in seven clinical treatments groups from an 3534-sample breast cancer cohort representative of population-based disease in the South Swedish healthcare region.

Patients and methods: Between 2010-01-09 and 2015-31-03, 5418 of 8591 patients with invasive primary disease in the healthcare region were included in the SCAN-B study (ClinicalTrials.gov ID: NCT02306096). Inclusion criteria included no generalized/prior contralateral disease and known surgery/treatment status (neo- or adjuvant). 3534 tumors were profiled by RNA sequencing and matched to clinicopathological patient data from the National Breast Cancer Register, with distribution of clinicopathological characteristics reflecting proportions in the catchment region. RNA profiles were classified according to 14 reported gene signatures featuring both GEPs (PAM50, IC10, CIT, TNBCtype) and specific risk predictors (e.g., Oncotype Dx, 70-gene, 76-gene, ROR-variants, genomic grade index). Classifications were investigated for association with patient OS by univariate and multivariate analyses in several adjuvant clinical treatment groups: TNBC-ACT (adjuvant chemotherapy, $n = 239$), TNBC-untreated ($n = 82$), HER2+/ER− with trastuzumab + ACT treatment ($n = 111$), HER2+/ER+ with trastuzumab + ACT + endocrine treatment ($n = 239$), ER+/HER2−/LN− with endocrine treatment ($n = 1074$), ER+/HER2−/LN+ with endocrine treatment ($n = 466$), ER+/HER2−/LN+ with endocrine + ACT treatment ($n = 447$), and ER+/HER2− untreated ($n = 201$).

Results: For the majority of signatures, analysis of classification demonstrated prognostic value limited to ER +/HER2- tumors given follow-up time. Several signatures (including Oncotype Dx, 70-gene, ROR-variants) showed strong predictive value in identifying a subset of ER+/HER2− patients receiving a combination of endocrine and ACT therapy with excellent overall-survival (> 96%), indicating appropriate therapy selection. In addition, for both ER+/HER2− treatment groups, signature analysis identified high-risk groups of patients in clear need of additional treatment beyond standard therapeutic regimes, even with less than 5 years of follow-up.

Conclusions: Our results support the prognostic association of gene expression signatures in large unselected population-based primary

breast cancer cohorts even with a short follow-up of OS. Importantly, prognostic associations are limited to specific subgroups for different classifiers and in population-based breast cancer some clinically important subgroups constitute a small proportion of cases. In this context, continued population-based inclusion and broad transcriptional profiling of breast cancer patients provides an opportunity for application to broader patient groups (e.g., TNBC and HER2+), and for consensus classification of individual risk assessments that could potentially provide more stable predictions.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P1.39

Low expression of Mediator Complex Proteins MED7 and MED23 is associated with poor prognosis in breast cancer

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Background: Mediator complex (MED) proteins have a key role in transcriptional regulation and some interact with the oestrogen receptor (ER). Interrogation of the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort suggested that the subunits 7 and 23 are negatively correlated with lymphovascular invasion (LVI; $p < 0.001$). Thus, MED7 and MED23 expressions were assessed in a large cohort of BC to determine its clinicopathological significance.
Methods: The prognostic impact of MED7 and MED23 gene expression was investigated in the METABRIC cohort ($n = 1980$) and externally validated using bc-GenExMiner v4.0. Immunohistochemical expressions of MED7 and MED23 were assessed in the Nottingham primary BC series ($n = 1255$). Associations with clinicopathological variables including LVI and patient outcome were evaluated.
Results: High MED7 mRNA and protein expressions were associated with good prognostic factors including low-grade, smaller tumour size, good NPI, ER/PR+ status, and negative LVI ($p < 0.05$). High MED23 mRNA expression was associated with low tumour stage and good prognostic integrative clusters 7 and 8 ($p < 0.001$). On IHC, higher nuclear-MED23 (n-MED23) expression correlated with low NPI, low-grade, older age, ER+ status, low Ki67 index and low N-cadherin expression ($p < 0.05$). Positive correlations with GATA3, STAT3 and CDC42 ($p < 0.001$) indicate possible interacting pathways. Low MED7 and n-MED23 protein expressions were associated with poorer breast cancer-specific survival (BCSS) within the whole cohort and ER+/luminal subgroup ($p < 0.05$). Pooled MED7 and MED23 gene expression data in bc-GenExMiner confirmed the association of low expression with poorer outcome and corroborates with the protein expression ($p > 0.05$).
Conclusions: Our study identifies MED7 and n-MED23 as novel prognostic markers in BC with relatively lower expression in poor prognostic categories.

P1.40

Breast Cancer Now Tissue Bank (BCNTB) bioinformatics

Breast Cancer Now

Breast Cancer Now Tissue Bank, London, UK

Introduction: The *Breast Cancer Now Tissue Bank (BCNTB) bioinformatics* aims to create an in silico environment with seamless connections to the in vivo and in vitro components of the *BCNTB*. *BCNTB bioinformatics* is available as an integrated research platform where breast cancer data can be shared, mined, integrated and analysed. Researchers have access to a centralised information gateway from which they can access a network of bioinformatic resources to query findings from publicly available data, from in-house data and from experimental data generated using BCNTB-supplied samples.
Results: Exploratory, investigative and interpretive analyses are available for transcriptomic, sequencing, genomic and mutation data generated using 8230 breast-related samples. These comprise specimens from tissues and cell lines generated by international cancer research efforts, such as The Cancer Genome Atlas and the Cancer Cell Line Encyclopedia, and individual research projects.
BCNTB:Analytics also provides researchers with the means to integrate and visualise discrete genetic events with continuous mRNA abundance data for user-defined genes. This multidimensional view of the data allows for the identification of alterations that co-exist within a sample and helps provide greater insight into the relationships between them. All results are presented in an interactive format so that researchers are able to focus and filter the results by areas of interest.

This module sets the analytical infrastructure that will host the experimental data derived from BCNTB samples. It is the unique interaction between BCNTB bioinformatics and its in vivo and in vitro counterparts that will ensure success in translating findings into benefits for patients.
Conclusion: *BCNTB bioinformatics* has been designed to allow data sharing, discoverability and re-usability. This platform is not a bioinformatics silo but rather a niche within the BCN tissue banking ecosystem that offers an unparalleled opportunity to add informative layers of molecular data to existing patient data available from the Bank.
BCNTB bioinformatics can be accessed at http://bioinformatics.breastcancertissuebank.org

PATHOLOGY

P2.1

Systematic review of breast lesions of uncertain malignant potential (B3 lesions) and their risk of malignancy

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Borderline breast lesions (B3 lesions) can coexist with malignancy. The magnitude of this risk varies between studies/lesion subtypes. This systematic review of the literature determines an accurate estimate of the risk of invasive/in situ malignancy identified by surgical excision biopsy, following diagnosis of a B3 lesion at core biopsy, within each B3 lesion subtype, guiding risk stratification/improving management strategies.

Literature searches (MEDLINE, Embase, HMIC, Scopus and Web of Knowledge), identified relevant studies between 1980 and 2015. Literature appraisal, meta-analysis and subgroup analysis performed to determine malignancy risk for all subgroups of B3 lesions (Papilloma, Radial Scar, AIDP, Lobular Neoplasia and FEA).

Searches returned 2289 citations, with 11 identified from other sources. Duplicate, unsuitable articles and abstracts/posters/reviews were excluded leaving 183 records. From these, 54 full text articles did not meet inclusion criteria. Meta-analysis was performed from 129 studies. Rates of malignancy varied from 6% in a radial scar with no atypia, to 32% for a papilloma with atypia. Malignancy upgrade rates between atypical and non-atypical lesions were statistically significant ($p < 0.05$). Study heterogeneity could not be explained by differences in core biopsy size or year of publication, however, a significant difference in upgrade rates to malignancy was observed between the US and non-US literature.

Many studies have assessed the risk of malignancy following diagnosis of B3 lesions, but are often small and lack statistical power. This is a comprehensive, inclusive assessment of available literature, on which to base-tailored management strategies.

	Number of included studies	Number malignant lesions	Total Number Lesions	Rate of malignancy and 95% CI (%)	Higgins I^2 (%)
Papilloma	42	351	2278	12 (10–15)	77.2
No atypia	14	90	1162	7 (4–10)	62.0
Atypia	11	91	298	32 (23–41)	57.4
ADH/AIDP	47	1114	4031	28 (24–31)	81.6
Radial scar	15	88	934	8 (6–11)	44.3
No atypia	4	22	334	6 (2–13)	72.8
Atypia	2	8	43	18 (8–32)	90.9
Lob. Neoplasia	38	345	2014	17 (13–21)	80.9
ALH	16	54	463	12 (5–21)	72.9
LCIS	16	76	359	22 (14–31)	63.6
FEA	19	179	1413	11 (8–14)	42.6
All B3 lesions	129	2160	11423	17 (15–19)	86.2

P2.2

Papillomas—impact of the new UK B3 guidelines on lesion management

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Introduction: Recent NHS BSP management guidelines for B3 lesions recommend diagnostic excision for papillomas with atypia and large-volume biopsy (LVB) for those without, proceeding to diagnostic excision if further atypia present. Our routine practice is to offer all papillomas second-line LVB, following MDT discussion, with surgery if B5 and annual mammography/routine recall depending on the presence of atypia. We have evaluated the potential impact of these guidelines on our benign biopsy rate.
Methods: Papillomas diagnosed between 01/2012 and 12/2016, recording LVB outcome and subsequent investigations.
Results: 103 papillary lesions identified over 5 years.
Ninety-six papillomas without atypia; LVB upgraded 3 to B5 and 13 to B3 atypia. 5/13 of the upgraded lesions underwent excision biopsy (all benign).

Seven papillomas with atypia; one LVB identified DCIS. Six diagnostic excisions, three identified DCIS, three benign.

Two cancers developed during surveillance, remote from index papilloma; one B5a after 1 year, and one B5b after 5 years.

Currently, 11 diagnostic excisions identified three cancers, LVB identified 4. With new guidelines, 20 diagnostic excisions would identify four cancers and LVB 3.
Conclusion: Seven percent of papillomas were subsequently upgraded to B5. Surgery for papillomas with atypia identified DCIS in 50%, however, LVB could improve pre-operative diagnosis in this group. Diagnostic excision biopsies for LVB atypia upgrades were benign, and no progression to cancer during follow-up occurred. New B3 guidelines would double our diagnostic excision biopsies for papillomas and reduce our pre-operative diagnosis rate. Second-line LVB is a safe, effective management strategy for all papillomas.

P2.3

What lessons can be learned from reviewing outcomes from pathological sampling of prophylactic mastectomy specimens? A retrospective analysis to inform best practice guidelines

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Currently there are no evidence-based guidelines for pathological sampling of prophylactic mastectomies (PM) performed on women deemed "at risk" of breast cancer due to cancer in the contralateral breast; BRCA1 or BRCA2 mutations or a significant family history. Guidelines from the Royal College of Pathologists (RCP) give general recommendations, advising specimens "be sampled more thoroughly than cosmetic reductions" stating visual inspection, manual palpation and slicing between 5 and 10 mm of thickness be performed with a minimum of two tissue blocks.

The literature is scarce, most papers endorse a similar approach to the RCP; Bernadette et al. suggest taking three random samples from

each quadrant and the nipple. Goldflam et al. advise the same with addition of samples from the central upper and lower breast.

There has been debate about whether examination of PM specimens leads to clinically significant findings. Literature is divided with two studies suggesting that it is of benefit and two finding it is of little benefit. With the advent of improvements in radiological imaging techniques, including MRI, further studies are needed to confirm whether specimen analysis is of benefit.

We analysed histopathology reports for patients who underwent PM in 2010–2016. Data collected included the presence of atypia, in situ and invasive disease, block number and what prior imaging was performed.

Preliminary analysis of 339 cases identified one unsuspected finding (cancer) not seen at macroscopic examination (ME) that would have changed management, a further two cancers but these were seen at ME, 4 cases of low volume DCIS and 20 cases of LCIS or atypia that would have not changed patient management. The average number of tissue blocks taken across the cohort was 12; the samples with findings had a higher average of blocks taken at 16. However, in conclusion, random sampling of macroscopically normal PM specimens picked up findings that would change patient management in 1 case (0.3%), suggesting that the College's recommendation of two blocks in macroscopically normal PM is adequate.

P2.4

Classical Lobular Carcinoma In Situ with Comedo Necrosis—a UK Multi Institutional Series

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Introduction: Classical lobular carcinoma in situ (LCIS) with comedo necrosis (also called mass forming LCIS, Florid LCIS) is a rare, recently described, variant of LCIS. It lacks marked nuclear atypia of pleomorphic LCIS (PLCIS). The presentation and natural history of the disease are unknown.
Methods: Cases from eight large specialist breast units including core biopsy and surgical excisions were identified and reviewed. Comprehensive imaging, histological and follow-up data were collected.
Results: A total of 28 cases, all e-cadherin negative, were identified. The mean age at diagnosis was 57.8 (range 42–85 years). Twenty cases presented with mammographic calcifications, seven with stromal deformity, and one as a mass lesion.
The B coding included B3, B4, B5a for lesions without invasion. 6 cases were misdiagnosed on core biopsy as DCIS (B5a).

On final histology, the lesion was associated with DCIS/PLCIS/invasion in 15 cases (53.6%) of which four were node positive. The invasive carcinomas were lobular (nine cases) and ductal (two cases). One patient had two tumours (ductal and lobular).

Fourteen pure lesions were diagnosed on core biopsy. On subsequent excision; two showed associated invasive ductal carcinoma, one DCIS, one DCIS and PLCIS and one showed DCIS only. The overall rate of upgrade to DCIS/PLCIS or invasive carcinoma for pure lesions was 35.7%.

3 patients (all with invasive disease) died and one patient developed disseminated lobular metastasis after 91 months of follow-up. All other 24 patients were uneventful.
Conclusion: This is the largest study to date of this rare, diagnostically challenging, entity. The lesion presents at an older age compared with classical LCIS. Mammographic calcification is the main presentation. The lesion was associated with DCIS/PLCIS or invasive carcinoma in 53.6% of cases. If diagnosed in isolation on core biopsy, the upgrade rate is 35.7%. The data indicate that this lesion is more aggressive than classical LCIS and support categorising as B4 on core biopsy as per current UK guidelines. Further prospective data collection is warranted to inform management.

P2.5

Dissecting Immune and Molecular Heterogeneity in Indian Triple-Negative Breast Cancer—A population with a higher percentage of Triple-negative breast cancer

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Background: Indian Triple-negative breast cancers (TNBC) comprise a substantially higher proportion of breast cancers than in developed countries (approximately 30 vs. 15–20%, respectively)[1,2]. Hence, effective management of TNBC requires identification of molecular factors that drive the heterogeneity including immune genes. Our goal is to perform a comprehensive analysis of the molecular and immune landscapes of TNBCs in India and compare it to the Western population.
Methods: A well-characterized cohort of 42 Indian breast cancer patient samples were used for this study. Unsupervised consensus clustering of gene expression and microRNA profiles was performed to identify TNBC subtypes and was compared with that of the METABRIC data. The subtypes were associated with mutations, immune cell enrichment and clinical outcomes.
Results: Our results showed at least three immune-related subtypes (two immune-enriched and one immune-dormant) of Indian TNBC. We arrived at a signature of 25 genes of which certain genes expressed in immune-enriched groups were related to cells from B, CD4 T, natural killer, macrophage, and monocytes. Interestingly, 57% of the dense infiltrated tumors overlapped with the one of the immune-enriched subtype which showed a better survival. We validated these subtypes using METABRIC data and observed significant differences in overall-survival ($p = 0.01$). Interestingly, proportion of better prognosis tumors are lower in our series as compared to METABRIC (23 vs. 58%) indicating greater proportion of more aggressive phenotype of Indian TNBCs.
Secondly, of a panel of MIRs profiled, we identified MIR155 to be significantly different in the above three immune-related groups with the immune-enriched group showing a higher expression of MIR155 ($p = 0.0001$) which could mean a crucial regulator of immune response in TNBC. This finding was also true of the METABRIC series.
Conclusion: Overall, this is the first study, to our knowledge, to profile large-scale Indian TNBCs and identify heterogeneity associated with disease prognosis and immune response. Future study warrants the validation and clinical utility of this classification to guide the subtype-specific therapy in Indian patients with TNBC.

P2.6

Automated nuclear grading of Ductal carcinoma in situ

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Understanding the evolution and ecology of cancer depends on characterizing the neoplastic cells and their microenvironments. We have developed automated image analysis methods of standard pathology H&E slides in order to quantify and study cancer ecology. We are applying these methods to ductal carcinoma in situ (DCIS) of the breast. DCIS is a common, preinvasive lesion with relatively low probability of progression to invasive cancer. The major challenge in the clinical management of DCIS, like most other preinvasive tumours, is prediction of progression and recurrence. High nuclear grade is often used as a prognostic factor of local recurrence. However, grading criteria are highly subjective and, hence, inter- and intra-personal variation of grading is commonly observed. We present an automated image analysis framework of H&E slides which analyses the morphology and texture of all epithelial cells at single-cell resolution within DCIS to identify the morphological variability present within the tissue sample. The method can then grade the DCIS region as high or low nuclear grade. After training on 15 samples, testing on 41 whole-slide images gave 85.4% agreement with at least one of two pathologists' grades. In comparison, the two pathologists agreed on grade in 73.8% of independently evaluated cases.

We demonstrate how automated histology image analysis generates clinically relevant grading for DCIS. Further study of spatial heterogeneity of tumour morphology based on the proposed framework will shed light on the evolution of cancer nuclear heterogeneity during DCIS progression to invasive cancer and influence on this evolutionary and ecological process from surrounding stromal cells.

P2.7

Thioredoxin-Interacting Protein (TXNIP) is an independent prognostic factor in breast ductal carcinoma in situ (DCIS)

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Background: Current clinicopathological parameters are useful predictors of recurrence in breast ductal carcinoma in situ (DCIS) but they are insufficient to reflect its molecular heterogeneity. The addition of biomarkers predicting recurrence has the potential for individualising therapy for DCIS. Thioredoxin-interacting protein (TXNIP), located on 1q21.1, shows copy number alteration in DCIS progressing to invasive disease and is a key player of oxidative stress. This study aims to assess the role of TXNIP in DCIS progression.
Patients and methods: 1057 consecutive DCIS, prepared as tissue microarrays, from patients treated in Nottingham between 1990 and 2012 were assessed for TXNIP expression by immunohistochemistry (IHC). Expression of TXNIP was correlated with patients' information, treatment and follow-up data.
Results: In pure DCIS tumours, low cytoplasmic TXNIP expression was associated with features of aggressiveness including high nuclear grade ($p = 1.6 \times 10^{6}$), presence of comedo necrosis ($p = 0.001$) and solid histological type ($p = 1 \times 10^{-6}$). Univariate outcome analysis showed an inverse association with development of local invasive and DCIS recurrence ($p = 0.04$). Multivariate analyses indicate that independent predictors of DCIS recurrence were low TXNIP expression ($p = 0.005$, HR 0.51 and 95% CI 0.32–0.81), larger DCIS size and high nuclear grade. Pure DCIS tumours showed higher TXNIP expression than DCIS associated with invasive breast cancer (IBC) ($p < 0.001$). Within the DCIS/IBC mixed cases; TXNIP expression was higher in DCIS component than in invasive component ($p < 0.001$).
Conclusion: TXNIP expression predicts local recurrence in DCIS patients. As an oxidative stress mediator, it could be used as a potential biomarker for prognostic stratification and management decisions.
Key words: DCIS, outcome, progression TXNIP, immunohistochemistry.

P2.8

Aurora Kinase A (AURKA) is an independent predictor of recurrence in breast ductal carcinoma in situ (DCIS)

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Background: Current clinical and pathological parameters are important predictors of recurrence in breast ductal carcinoma in situ (DCIS) but they are insufficient to reflect its molecular heterogeneity. Biological characterisation has the potential for individualising therapy for DCIS. Aurora Kinase A (AURKA), located on 20q13.2, shows copy number alteration in DCIS and is a key regulator of cell cycle progression. High expression of AURKA is associated with poor outcome in invasive breast cancer (IBC); however, it is not confirmed in the pre-invasive stage. This study aims to assess the role of AURKA in DCIS behaviour.
Patients and methods: One thousand and fifty-seven consecutive DCIS patients treated in Nottingham between 1990 and 2012 were prepared as tissue microarrays. Patients' clinical information, management and follow-up data were retrospectively collected. The expression of AURKA was assessed immunohistochemically and assessed with clinicopathological parameters.
Results: Pure DCIS lesions showed higher expression of AURKA compared to lesions associated with IBC ($p = 3 \times 10^{-8}$). In pure DCIS tumours, high nuclear AURKA expression was associated with features of aggressiveness including high nuclear grade ($p = 0.04$), presence of comedo necrosis ($p = 0.03$), solid and micropapillary histological types ($p = 0.005$ and $p = 0.012$, respectively). Univariate outcome analysis showed positive association with development of local invasive recurrence ($p = 6 \times 10^{-6}$). Multivariate analyses indicate that independent predictors of DCIS recurrence are high AURKA expression ($p = 8 \times 10^{-5}$, HR 5.5 and 95% CI 2.4–12.9), larger DCIS size and high nuclear grade.
Conclusion: AURKA expression predicts local recurrence in DCIS patients and is potentially useful in prognostic stratification of DCIS patients for management decisions.

Key words: DCIS, outcome, recurrence, AURKA, immunohistochemistry

P2.9

Loss of DNA polymerase beta (POLβ) is associated with invasion in Ductal Carcinoma in situ (DCIS)

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Introduction: The prediction of clinical behaviour of Ductal Carcinoma in situ (DCIS) of the breast to develop into invasive breast cancer (IBC) remains inadequate. Genetic variations in DNA repair genes is associated with risk of developing IBC. We hypothesise that markers of base excision DNA repair may provide possible prognostic roles to predict DCIS progression to invasive disease. The aim of this study is to characterise DNA polymerase B (POLβ) protein expression in DCIS and to determine its association with progressive disease.
Methods: A cohort of 831 cases of pure DCIS and 239 cases of mixed DCIS with IBC were prepared as tissue microarrays. POLβ protein expression was assessed using immunohistochemistry and correlated with clinicopathological parameters and patient outcome.
Results: POLβ was highly expressed within the pure DCIS (81.3% of cases) and also DICS associated with IBC (87.5%) In the pure DCIS cohort, low expression of POLβ correlated with high nuclear grade ($p < 0.001$), comedo type of necrosis ($p = 0.001$) negative ER and PR status ($p < 0.001$). In the mixed cases, lower expression of POLβ is seen in invasive component than in DCIS component ($p < 0.001$), however, no significance was seen with patient outcome.
Conclusion: These results suggest that loss of *POL*β expression is associated with aggressive DCIS.
Relevance to patients: New discovery of predictive markers of DCIS progression will help to tailor patient management.
Keywords: DCIS, Breast cancer, DNA polymerase beta (*POL*β), DNA damage response.

P2.10

Deep Learning-based detection of Ductal carcinoma in situ in breast cancer histology images

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Ductal carcinoma in situ (DCIS) is a known precursor of invasive breast cancer (IBC). Its diversity in morphology, shape and size poses a significant challenge for automated identification in digital pathology. Recently, we demonstrated that lymphocyte spatial patterns identified in histology slides are clinically relevant in ER+ IBC (Heindl et al. JNCI 2017). However, spatial heterogeneity of lymphocytes in relation to DCIS in IBC is not well characterised.

In this work, we propose a deep-learning approach for automated DCIS spatial mapping in 1178 IBC haematoxylin and eosin (H&E) whole slide images from the TransATAC study. Context-specific preprocessing of epithelial regions is employed to increase the specificity of our DCIS detection pipeline. The proposed algorithm then separates the tumour epithelial regions from the stroma based on a shallow deep neural network. This is followed by a transfer learning model to fine-tune and detect DCIS regions. The model was trained using 3284 regions derived from pathologist delineation comprising of DCIS, normal duct, stroma and tumour regions. In the validation cohort, consisting of 232 regions, the model generated an accuracy and F-score of 0.89, and 0.90, respectively. The receiver operating curve for DCIS regions and tumour yielded 0.84 and 0.92, respectively.

Our approach presents a new way to identify DCIS components in H&E images of IBC. Further, it will enable the analysis of prevalence and clinical relevance of lymphocyte distribution near DCIS, which may lead to improved understanding and scoring for immune response in ER+ breast cancer.

P2.11

Stratification of patients treated for ductal carcinoma in situ of the breast (DCIS) according to risk of local recurrence

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Ductal carcinoma in situ (DCIS) are heterogeneous precursor, non-invasive breast lesions. Although only about 40% of these are likely to become invasive, we currently lack accurate tools to identify patients at higher risk of local recurrence or progression to invasive breast cancer (IBC) and most DCIS patients are treated by breast-conserving surgery (BCS), followed by whole-breast radiotherapy (RT) for high-grade DCIS. There is a growing concern for the issue of overtreatment and a pressing clinical need to move towards more personalised treatment.

We identified 466 patients (median age 61, range 35–94) with DCIS treated locally between 2000 and 2010. From these, we designed a single-institution case–control match series study comprising two cohorts:

- One hundred and fifty patients with low/intermediate-grade DCIS treated with BCS alone:
  - Thirty patients who have recurred;
  - One hundred and twenty who have not recurred by 10 years.
- One hundred and forty-six patients with high-grade DCIS treated by BCS + RT:
  - Twenty patients who have recurred;
  - One hundred and twenty six who have not recurred by 10 years.

Median follow-up was 7.3 years. Lexogen QuantSeq sequencing of the 296 patient cohort has been performed and in-depth analysis is currently underway and will be presented. This will be followed by RT-qPCR and IHC validation to confirm efficacy and determine the most cost-effective and reliable method. We have also identified an independent validation cohort ($n = 195$).

This is the first DCIS biomarker discovery study including whole-genome analysis and a matched cohort design to account for the effect

of RT. We aim to identify novel biomarkers and develop a clinically applicable prognostic test for robust stratification of patients according to their individual risk of recurrence after BCS ± RT. Such a tool would enable more effective management of DCIS, identifying high-risk patients likely to benefit from further therapy following surgery and low-risk patients for whom this may be safely omitted.

P2.12

A literature review: the role of plakoglobin as a biomarker to determine the invasive potential of ductal carcinoma in situ

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Background: DCIS is a heterogeneous disease exhibiting varying degrees of aggressiveness; approximately 40% of DCIS will progress into invasive cancer. Currently, no molecular marker is identified that can reliably predict the invasive potential of DCIS in an individual patient. Additionally, DCIS carries a recurrence rate of 3–17% within 10 years, and half of these recurrences could be invasive cancers. A biomarker that can reliably predict which DCIS lesions have a high likelihood of developing into invasive cancers can potentially prevent over- or undertreating patients.
Method: A search of electronic databases 'MEDLINE' and 'PUBMED' for relevant published articles was undertaken in February 2015. Publications deemed sufficiently relevant to the topic and published between January 1990 and February 2015 were included in the review.
Results: Desmosomes are molecular complexes that attach adjacent epithelial cells together by means of linking proteins together. Any disruption in desmosomal proteins can lead to certain diseases—such as cardiomyopathy and pemphigus and cancer progression. Plakoglobin (PG) is a desmosomal protein that has been implicated in malignant transformation associated with phenotypic features of reduced cell–cell adhesion, increased invasiveness, migration and cell proliferation. PG can be a potential biomarker for cancer progression, differentiating between chronic pancreatitis and pancreatic ductal adenocarcinoma (PDAC) cancer. *Ellis* et al. have reported reduced PG expression in invasive Paget's disease of the vulva, when compared to intraepidermal Paget's disease of the vulva.
Conclusions: The loss of PG can serve as a potential biomarker to predict the invasive potential of DCIS. If PG is a reliable predictor of the invasive potential of DCIS lesions, this will help to tailor treatment for patients with DCIS according to its invasive potential. For example, patients with low-grade DCIS with a low invasive potential will not have to undergo disfiguring surgical treatment of mastectomy.

P2.13

Myoepithelial cell phenotype in DCIS progression: Functional significance of integrin αvβ6 and fibronectin

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Introduction: Progression to invasive breast cancer follows transition through a pre-invasive stage, ductal carcinoma in situ (DCIS). Unlike invasive disease, DCIS has not yet invaded or may never invade into the surrounding tissues. With the inability to determine which cases will and will not progress, there are concerns of overtreatment. There is a need to identify robust prognostic markers to better direct therapeutic intervention, and focus has turned to 'normal' host cells to identify such markers. Normal myoepithelial cells (MECs), which form the interface between the epithelium and stroma, exert a tumour-suppressor function. In DCIS, MECs are altered with de-novo expression of integrin β6 and up-regulation of Fibronectin (FN). This study aims to evaluate the clinical and functional relevance of these changes.
Methods: Samples of DCIS and DCIS with invasion were analysed for the expression of b6 and FN by immunohistochemistry. Primary MECs and established MEC lines, with (β6-1089) and without (N-1089) β6 expression were used to investigate the mechanisms regulating the expression, and function of these molecules.
Results: Expressions of β6 and FN are significantly associated with progression of DCIS to invasion. β6-1089 up-regulates FN and FN isoforms, EDA and EDB, at both the protein and mRNA level, compared to N-1089. Expression of FN promotes β6-mediated TGFβ activation and signalling, and breast cancer cell invasion in vitro. The tumour-promoting effect of β6-1089 is enhanced by up-regulation of MMP13.
Conclusion: Expression of both β6 and FN by MECs is required for TGFβ activation and signalling, which promotes breast cancer cell invasion. These changes may be used to determine which DCIS lesions will and will not progress, allowing for more robust patient stratification.

P2.14

Deciphering the molecular landscape of E-cadherin in ductal carcinoma of the breast by next-generation sequencing

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Background: E-cadherin, an adhesion molecule involved in variety of cellular functions, is a tumour suppressor gene in lobular breast cancer but not in ductal BC (IDC). However, a proportion of high-grade IDC shows loss/reduced E-cadherin expression. This study aims to apply Next-Generation Sequencing (NGS) to a cohort of IDC with a well-characterised E-cadherin expression status to assess the molecular profiles of these tumours and potentially decipher the mechanisms and impacts of E-cadherin loss in IDC.
Methods: E-cadherin protein expressions was determined using tissue microarrays ($n = 1500$). Results were validated on the selected subgroup of high-grade triple-negative IDC using full face sections. RNA-seq analysis was completed on 106 patients sample containing E-cadherin positive ($n = 58$) and negative/low ($n = 48$) IDC using the HiSeq 2500 instrument (Illumina, Inc.) The targeted read count was 60 M total reads per sample. Row FASTQ sequence reads files were quality and adapter-processed using the trim galore wrapper for FASTQC and Cutadapt, and the resultant QC reads were aligned to the hg38 (GRCh38.P5) built for the human genome using the Atlas aligner. Differentially expressed genes/transcripts with respect to

E-cadherin status were assessed using Robina implementation of Edge-R.
Results: Differential Gene Expression (DGE) by NGS identified a total of 2132 gene (Benjamin–Hochberg; $p < 0.05$, differentially expressed by more than two-fold, false discovery rate was < 0.05). IDC with reduced E-cadherin expression showed dysregulation of genes regulating Wnt signalling pathway (FZD2, GNG5, HLTF, WNT2, and CER1), PIK3-AKT signalling pathway (FGFR2, GNF5, GNGT1, IFNA17, and IGF1) and other relevant pathways involved in carcinogenesis. Importantly, key genes reported to be differentially expressed between ductal and lobular tumours did not show association with E-cadherin loss in IDC.
Conclusion: Study revealed application of high throughput molecular techniques in deciphering complex biological phenomena. E-cadherin loss in IDC appears to be a part of the genomic instability occurring in the later carcinogenesis status rather than an initial neoplastic event as in lobular carcinomas.

P2.15

Myoepithelial cell-associated galectin-7: functional and clinical relevance in DCIS progression

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Background: A review of breast screening has highlighted the need to reduce overdiagnosis. Ductal Carcinoma In Situ(DCIS) contributes significantly to this. As DCIS evolves there are concomitant changes in the ductal microenvironment. Previously we have identified changes in the non-neoplastic myoepithelial cells that convert these tumour-suppressor cells to tumourpromoters. This study investigates the functional and clinical significance of myoepithelial-associated Galectin-7, a protein shown to have anti-apoptotic function, and to use this to develop a biomarker panel to risk stratify DCIS.
Methods: The expression of Galectin-7 was assessed by immunohistochemistry in 2 groups of samples: pure DCIS (low-risk model) and DCIS with associated invasion (high-risk model). Each Individual DCIS duct was scored as positive, heterogeneous or negative for Galectin-7.
Normal primary myoepithelial cells isolated from reduction mammoplasty were used as a model to investigate the function of Galectin-7. These cells endogenously express high levels of Galectin-7. Galectin-7 was silenced using siRNA and apoptosis assessed using cleaved PARP and caspase-3. Phosphoproteomics and RNA-sequencing have been undertaken to analyse the global impact of Galectin-7 loss.
Results: 1926 DCIS ducts were scored for immunohistochemical expression of Galectin-7. Pure DCIS and DCIS with invasion had 338 and 144 positive DCIS ducts, respectively ($p = 0.0014$). Pure DCIS and DCIS with invasion had 99 and 646 negative DCIS ducts, respectively ($p = 0.0002$). The remaining ducts were heterogeneous. Significant knockdown of Galectin-7 was achieved in primary myoepithelial cells. Western blotting demonstrated increased expression of cleaved PARP and caspase-3 in Galectin-7 knockdown cells, indicating reduced Galectin-7 increases apoptosis.
Conclusion: The loss of myoepithelial Galectin-7 in DCIS is associated with a more aggressive DCIS phenotype. Culture models indicate loss of Galectin-7 promotes apoptosis, suggesting this may contribute to disease progression.
Galectin-7 shows translational promise in the development of an immunohistochemistry panel in the prediction of DCIS prognosis and subsequently reducing overdiagnosis. Galectin-7 is currently being validated using the UK DCIS Trial.

CLINICAL EARLY DISEASE

P3.1

Male Breast Cancer: Our 15-year experience

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Introduction: Breast cancer is rare in men, comprising less than 1% of all breast cancers and less than 1.5% of all malignancies in men. The diagnosis of male breast cancer (MBC) is often delayed, resulting in poor outcome.
Methods: We undertook a retrospective study of all cases of MBC treated in our unit between June 2002 and June 2017. Their demographics, histopathology, treatment and survival data were reviewed.
Results: Twenty-six cases of MBC were identified during the 15-year period. One patient had simultaneous bilateral cancers. Median age was 66 (41–91) years, and median follow-up was 33 (3–84) months. Mean tumour size was 26 (13–70) mm. Histology of tumours: 22 invasive ductal carcinoma, two DCIS and two encysted papillary carcinoma. Three patients had concurrent Paget's disease of the nipple. Twenty-four patients with known oestrogen receptor (ER) status were all positive. Only two MBC were HER2 positive.
The common presenting symptoms were palpable lump, nipple bleeding and nipple changes. Twenty patients underwent mastectomy and axillary surgery. Three patients only had primary endocrine therapy. Twelve patients received radiotherapy and eight had chemotherapy. Axillary nodal metastases were present in ten cases, and seven patients had distant metastases. Seventeen patients remained disease-free. Two patients developed recurrence. Five patients have succumbed to MBC, and three others have died due to unrelated medical co-morbidities. BRCA2 mutation was present in one case.
Conclusion: The mean age at diagnosis for MBC was higher when compared to female patients. All our MBC were ER positive. Treatment regime for MBC was similar to female breast cancers. Survival of MBC is comparable to the female counterparts.

P3.2

PeriOperative Endocrine Therapy for Individualised Care-2 trial: results of a feasibility phase

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Background: The PeriOperative Endocrine Therapy for Individualised Care-2 (POETIC-2) trial will aim to match gene expression

signatures with targeted agents against endocrine resistance in early breast cancer patients with a high risk of recurrence, as predicted by high Ki67 proliferation marker after 2 weeks' aromatase inhibitor (AI) therapy.
Aims: (i) To determine feasibility of patient recruitment, including acceptance of an additional core biopsy at 2 weeks in a 4-week presurgical trial modelling POETIC-2; (ii) To determine the feasibility of performing Ki67 and gene expression analyses in time to deliver targeted therapy preoperatively.
Methods: 20 postmenopausal patients with ER+ breast cancer were prospectively enrolled at two centres. Patients received 4 weeks' presurgical AI. Tumour biopsies were taken at baseline (day 0) and surgery (day 28) from all patients. A day-14 biopsy was taken from patients with a baseline Ki67 $\geq$ 10% and these patients received the targeted agent fulvestrant. Ki67 was measured by immunohistochemistry and gene expression was evaluated by NanoString nCounter system.
Results: Patients were recruited over 15 months. Overall 51% of eligible patients approached consented to the study and 21% declined due to the need for an additional biopsy at 14 days. Sixteen patients presented baseline Ki67 $\geq$ 10% and therefore received fulvestrant treatment. Baseline Ki67 and gene expression analyses were performed and results were available within 2 weeks for all patients. Within 1 week, baseline Ki67 was performed for 85% of patients and gene expression for 35%. Gene expression analysis failed in two samples due to low yield/quality of extracted RNA
Discussion and conclusions: Since the launch of the feasibility study, the design of POETIC-2 has changed to a 6-week study. However, the lessons from the feasibility study remain fully relevant and reveal that it is feasible and Ki67 and gene expression analyses can be performed in time to deliver targeted therapy.

P3.3

National analysis of recurrence after early breast cancer in Scotland

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Background: Recurrence rates are important clinical outcomes that have been difficult to capture using routine administrative data in the UK. The clinical community in Scotland has called for a broad set of outcomes to form part of the National Cancer Quality audit programme. This feasibility project, for the first time, reports recurrence-free survival after early breast cancer in the national Scottish population.
Methods: Patients with surgically treated early breast cancer diagnosed in 2007 and 2008 were identified from national records. Clinical records were scrutinised by audit staff in all 14 Health Boards. Recurrence-free survival was estimated using the Kaplan–Meier method. Co-variate adjustment for age, stage, ER/HER2 status, mode of detection, radiotherapy, chemotherapy and socioeconomic status used the Cox method. The ability of the "NHS Predict" tool was assessed for its ability to predict recurrence and mortality.
Results: In total, 3831 patients were included. Median age at diagnosis was 60. Five-year disease-free survival was 83.6% (95% CI 82.3–84.8). Local, locoregional and distant invasive recurrence rates at 5 years were 2.4, 3.0 and 9.3%, respectively. In multivariate analysis, significant predictors of recurrence included nodal status, ER status, mode of detection, tumour size, age and socioeconomic status. In the 1914 patients treated with breast conserving surgery, the local recurrence rate was 2%. A 1% increase in predicted mortality was associated with a 2.6% increase in the risk of recurrence. An estimated 100 man-hours per health board was required to capture these data.
Conclusion: It is demonstrated as feasible to collect recurrence outcome data at a Scottish national level. The required time for manual audit may not be sustainable and more efficient methods should be explored. The NHS Predict tool could be further developed to predict recurrence as well as mortality.

P3.4

The TeaM (Therapeutic Mammaplasty) Study: A National Audit of the practice and outcomes of therapeutic mammaplasty

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Introduction: Therapeutic mammaplasty (TM) which combines breast reduction and mastopexy techniques with tumour excision may extend the boundaries of breast-conserving surgery (BCS) and improve outcomes for patients but the prevalence of this operative option among surgeons in the UK and its success rates are unknown. The TeaM study is a multicentre prospective audit that aims to investigate the practice and outcomes of TM.
Methods: Patients undergoing TM between 1 September 2016 and 30 June 2017 were recruited to the study. Demographic, pre-operative, operative, oncological and complication data were collected. The primary outcome was unplanned re-operation for complications. Secondary outcomes included re-excision rates and time to adjuvant therapy.
Results: Eight hundred and eighty patients underwent 899 TM procedures at 51 centres. Approximately half were screen-detected ($n = 424$, 48.1%) cancers. The most common indications for TM were either to avoid the poor cosmetic outcome associated with standard BCS ($n = 708$, 78.6%) or a mastectomy ($n = 382$, 42.4%). Wise-pattern skin incisions were the most common ($n = 429$, 48.0%) with the majority of procedures preserving the nipple ($n = 798$, 88.8%) most often using an inferior ($n = 204$, 25.5%) or superomedial ($n = 201$, 25.2%) pedicle or a central mound technique ($n = 225$, 28.1%). Contralateral symmetrisation was performed in approximately one-third of cases ($n = 284$, 32.2%). Median lesion size was 24 mm (IQR 16–38 mm). Incomplete excision was seen in 134 cases (14.9%) with 54 (6.1%) patients ultimately requiring a mastectomy. 181 (20.1%) TMs experienced a complication but these were often minor with just 15 (1.7%) patients requiring re-operation for complications. Median time to start of adjuvant therapy was 54 days (IQR 42–66).
Conclusions: Therapeutic mammaplasty is a safe and effective alternative to mastectomy or standard BCS. Further work is now required to explore the impact of the procedure on quality-of-life and determine the optimal timing of symmetrisation surgery.

P3.5

Palbociclib Dosing Audit

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Background: The CDKs are a large family of serine–threonine kinases that play an important role in regulating cell-cycle progression. As such, recently these drugs have begun to be used as standard practice in the treatment of HR-positive breast cancers.
The addition of Palbociclib to standard first-line endocrine treatment with Letrozole or Fulvestrant for post-menopausal HR+ almost doubled length of overall-free survival.

Provided current guidelines are closely adhered to with respect to appropriate dose reduction and/or dose delay for neutropenia, there is no impact of dose change on efficacy in the clinical trials which have been published.
Method: The audit carried out during Quarter 3 of 2017 assessed the dosing of Palbociclib against IBRANCE guidelines, and the usage of GCSF for patients treated at Leaders in Oncology Care between April 2016 to April 2017. After excluding any Extended Access Programme patients, 47 patients were included in the audit.
Results: Twenty-six patients received Palbociclib + Letrozole and 21 received Pablociclib + Fulvestrant. There were no incidents of grader 3 anaemia or thrombocytopenia resulting in dose reduction or delay (the IBRANCE guidelines advise dose reduction above grade 2 if no recovery in more than 1 week).16 patients continued treatment without any dose reduction over 6 months. Twelve patients received GCSF at least once during their treatment. Two patients who required no reduction or delay had received GCSF at during treatment.

P3.6

Randomised, phase-II/III trial to evaluate the safety and efficacy of the addition of olaparib to platinum-based neoadjuvant chemotherapy in triple-negative and/or germline BRCA-mutated breast cancer patients

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Background: No specific targeted therapies are available for Triple-Negative Breast Cancers (TNBC), an aggressive and diverse sub-group. The basal TNBC sub-group share some phenotypic and molecular similarities with germline BRCA (gBRCA). In gBRCA patients, and potentially other homologous recombination deficiencies, these already compromised pathways may allow drugs called PARP inhibitors (olaparib) to work more effectively.
Aims: To establish if the addition of olaparib to neoadjuvant platinum-based chemotherapy for basal TNBC and/or gBRCA breast cancer is safe and improves efficacy (pathological complete response (pCR)).
Trial design: Three-stage open-label randomised phase-II/III trial of neoadjuvant paclitaxel and carboplatin ± olaparib, followed by clinicians' choice of anthracycline regimen. Stage 1 and 2: Patients are randomised (1:1:1) to either control (3 weekly carboplatin AUC5/ weekly paclitaxel 80 mg/m2 for 4 cycles) or one of two research arms with the same chemotherapy regimen but with two different schedules of olaparib 150 mg BD for 12 days. Stage 3: Patients are randomised (1:1) to either control arm or to the research arm selected in stage 2.
Methods: Stage 1: Safety (completed Aug 2017, results available Q1 2018); Stage 2: Schedule selection criteria: pCR rate and completion rate of olaparib protocol treatment. Using a "pick-the-winner" design there is 90% power, 5% one-sided significance level to test null hypothesis of pCR $\leq$ 35% versus an alternative hypothesis of pCR $\geq$ 55% in each research arm. Stage 3: Efficacy: anticipated pCR $\sim$ 55–60% for all trial patients and $\sim$ 60–65% for gBRCA patients. The trial is powered to detect an absolute improvement of 15% (all patients) and 20% (gBRCA patients) by adding olaparib to chemotherapy (enriched design). TNBC patient recruitment will be capped, to ensure required gBRCA patients are enrolled. Enrichment design is applied with overall significance level $0.05(\alpha) = 0.025(\alpha\text{all}) + 0.025(\alpha\text{gBRCA})$ and 80% power. Target accrual: 527 [gBRCA 220] Current accrual on 28th Sep 2017: 96; Sites activated: 17 [expected number of sites 30–50].

P3.7

Risk of chemotherapy-related amenorrhoea (CRA) in premenopausal women undergoing chemotherapy for early breast cancer

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Background: Many women diagnosed with early-stage breast cancer (BC) will undergo temporary or permanent CRA. While the rate of CRA varies with patient age and chemotherapy regimens administered, few studies have investigated the impact of other clinicopathological factors.
Methods: This is a retrospective analysis of a cohort of pre-menopausal women ($n = 112$) with early BC who received either FEC-T or FEC75 as part of their treatment at King's College hospital between 2005 and 2010. Clinicopathological data were collected and analysed using χ^2 statistics.
Results: At the time of treatment, the mean age was 43 years (range 35–50). Of the 107 women treated, 81 (76%) developed CRA, all of whom had either regular ($n = 66$, 82%) or irregular ($n = 15$, 18%) menses prior to the commencement of therapy. Of the 26 women who continued menses on chemotherapy, 17 (66%) with regular menses at pre-treatment developed irregular menses on-treatment. After treatment, menses resumed in 32/81 patients with amenorrhoea (40%), 29 (91%) of which had regular menses prior to treatment. After treatment, 18 (22%) resumed regular and 14 (17%) irregular menses. In 107 patients, the incidence of irregular menses prior to treatment was found to be significantly higher in patients with ER-positive BC. Only age of menarche was found to be significantly associated with CRA ($p = 0.018$), with higher incidence in patients with a younger age of menarche onset. Age at the time of treatment was not significant. Post-treatment resumption of menses following the development of CRA was more strongly associated with younger age ($p < 0.0001$) and ER-negative BC\adjuvant tamoxifen untreated ($p = 0.003$). Chemotherapy regimen given, parity, smoking status, and history of previous gynaecological conditions were not found to be significantly associated with amenorrhoea, the resumption of menses or changes in regularity of menses before, during or after chemotherapy.

Conclusions: Few factors contribute to the variability of CRA in premenopausal women. Further studies to improve prediction of CRA, early menopause and impact on fertility are warranted.

P3.8

Best practice for multiple fibroadenomas: a critical literature review

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Objectives: Breast fibroadenoma is one of the most common benign breast tumours. A single fibroadenoma is easily manageable; however, expert panels have differed with respect to the recommended management of multiple fibroadenomas. Different approaches include conservative management in the form of annual follow-up, surgical excision of lesions and more recently a paradigm shift towards less invasive alternatives to surgery, such as interstitial laser therapy (ILT) and radiofrequency ablation (RFA). Current literature is unhelpful in terms of collating data on the short- and long-term success of these various approaches. This review examines interventional studies in order to evaluate the effectiveness of conservative, interventional and surgical measures in managing multiple fibroadenomas.
Methods: Medline, EMBASE, PubMed and Cochrane Library were searched, according to pre-specified selection criteria. Study quality was assessed using the Jadad scale.
Results: Of 364 search results, seven were eligible for review. Patients from six studies were actively treated either by surgery, ILT or RFA. All three of these approaches demonstrated a statistically significant reduction in nodule volume on follow-up, although this was reached far sooner (1-month post-intervention) with surgery and RFA. The surgically treated group seemed to have the highest complication rate (20%) and poorest cosmetic outcome.
Conclusion: From the aspect of therapeutic efficacy, the ablation and volume reduction rates of RFA are not inferior to traditional surgical resection. Furthermore, data points to a lower complication rate associated with RFA. The advantages of RFA as a management option are its therapeutic efficacy, low complication rate and good cosmetic outcome, which can maximally meet the aesthetic demands of patients. Newer surgical approaches such as the round-block technique remain important and have the benefit of allowing generous access to the breast while confining the incision to the areolar margins.

P3.9

Best practice for Benign Phyllodes Tumour: A Critical Literature Review

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Objectives: Expert panels have differed with respect to two aspects of the recommended management of benign phyllodes tumours (BPT): what the preferred approach to surgical excision is, and the necessity of obtaining negative surgical margins. Historically, wide local excision (WLE) has been the favoured approach and re-excision for positive margins has been the standard of care. Recently, groups have used US-guided vacuum-assisted biopsy (US-VABB) to excise these lesions and have conservatively followed up patients with positive margins. Current literature is unhelpful in terms of collating the data on the recurrence rates among patients undergoing WLE verses US-VABB and re-excision verses observation. This review examines interventional studies in order to evaluate the most effective management protocol for patients with BPT.
Methods: Medline, EMBASE, PubMed and Cochrane Library were searched, according to pre-specified selection criteria. Study quality was assessed using the Jadad scale.
Results: Of 301 search results, nine were eligible for review. Seven studies compared the relapse rate between patients who had positive margins and patients with negative margins. All seven studies demonstrated no significant difference in disease recurrence (median follow-up time 41 months) between either group. The remaining two studies compared US-VABB and WLE as different approaches to tumour resection. Both studies demonstrated no significant difference in disease recurrence (median follow-up time 35 months) between either treatment group, although patients treated with US-VABB suffered less complications and had better cosmetic outcome.
Conclusion: Re-excision of BPT in order to obtain a negative surgical margin does not lower the risk of recurrence. US-VABB is as effective at removing BPT as WLE in terms of minimising recurrence, whilst also having a lower complication rate and better cosmetic outcome.
Considering the recurrence of benign disease is already low (5%), this all supports a conservative approach involving regular follow-up after US-VABB excision.

P3.10

Validation of an autoantibody blood test for the detection of early breast cancer (BC), particularly hormone receptor-positive BC

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Background: Breast cancer (BC) remains the most common type of cancer in women, with an incidence of 1.6 million cases per year worldwide. There is an urgent need to identify a test, preferably a blood test showing high sensitivity and specificity for early BC across all ages of patients and all tumour types.
Aim: To identify a panel of tumour-associated antigens (TAAs) which would detect AABs in the blood with high sensitivity and specificity for early BC: enabling cancer/normal discrimination.
Methods: Serum samples from 120 BC patients and matched controls were tested against a panel of 60 TAAs using an optimised new multiplex microarray platform.
Results: Using a panel of 12 TAAs, AABs were detected in pre-op blood of 34/60 (57%) primary BC patients compared to 9/59 controls (15%) ($p = 0.000003$); one control sample data were unavailable. This gave a sensitivity of 57% and specificity of 85%. There was no significant difference for AAB detection when compared to tumour size, grade, lymph node status or ER status. In the sub-group of 60 patients where ER antigen was measured using a panel of 8 TAAs, AABs were detected in 20/29 BC patients compared to 2/30 controls ($p = 3.5e\text{-}7$). This represents a sensitivity of 69% with a specificity of 93%.
Conclusions: These results confirmed our hypothesis that AABs can be detected in women of all ages with early BC. If a panel of AAB

assays can be validated, it opens the possibility of a blood test for detection of early BC.

P3.11

Acute toxicity and quality-of-life in breast cancer patients treated by radiotherapy in the multi-centre REQUITE cohort study

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Background: Although patient-reported outcomes (PROs) to assess health-related quality-of-life (QoL) are increasingly incorporated in radiotherapy trials, QoL in the acute period following treatment remains underreported. This study assessed the relationship between acute radiotherapy toxicity and QoL in breast cancer patients.
Methods: Breast cancer patients ($n = 2072$) were recruited following breast-conserving surgery across eight centres in Europe and North America into a multi-centre prospective cohort study (www.requite.eu). Treatment data, toxicity scored according to CTCAE v4.0 and PROs from EORTC-QLQ-C30 and –B23 were available for 1750 patients at baseline and on completion of radiotherapy. Association of acute toxicity endpoints (dichotomised) and worsening QoL ($\geq$ 10 point change from baseline) was investigated using multivariate logistic regression, adjusted for age, BMI, total radiotherapy dose, seroma, chemotherapy, tamoxifen, analgesic use, smoking and alcohol intake.
Results: By the end of radiotherapy, 24.2% of patients experienced $\geq$ grade 2 erythema and 31.6% $\geq$ grade 1 oedema, and 9.5% were affected by acute desquamation (skin loss). Global health status, fatigue, pain and breast symptoms worsened significantly compared to baseline. Acute erythema and acute desquamation were significantly associated with worsening breast symptoms (OR 1.71, 95% CI 1.41–2.06; and OR 1.77, 1.18–2.67), while acute erythema was also associated with worsening pain (1.24, 1.03-1.50). There was no significant association of any acute toxicity endpoint with worsening global health status.
Conclusions: Management of early toxicities that affect breast-specific symptoms and pain may improve QoL during radiotherapy. Overall QoL (global health status) during breast radiotherapy is likely to be influenced by a range of non-treatment factors.

P3.12

The LungSpare Study: A dosimetric comparison of radiotherapy techniques for right-sided breast cancer patients requiring treatment of the internal mammary chain

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Background: The use of internal mammary chain radiotherapy (IMC-RT) is increasing. Inclusion of the internal mammary chain (IMC) in the radiotherapy target volume results in an absolute increase in ipsilateral lung $V_{17\ Gy}$ (Volume of lung receiving 17 Gy) of approximately 10% (Offersen et al. 2014). For patients with left breast cancer, use of deep inspiratory breath hold techniques (DIBH) is standard within most departments in order to reduce heart doses. Dosimetric studies suggest that treatment in DIBH also reduces ipsilateral mean lung dose for right-breast-affected patients requiring IMC-RT (Poortmans et al. 2015). However, due to the increased linear accelerator time associated with use of DIBH, not all RT centres are able to adopt DIBH techniques for right-sided breast cancer patients requiring IMC-RT. An alternative approach to reducing lung dose in patients undergoing field-based IMC-RT is to displace the match-plane between the tangents and the nodal fields superiorly. This dosimetric study compares the lung-sparing ability of the superior match-plane move against that of a DIBH technique.
Method: Ten previously treated patients who had CT planning scans in DIBH and free breathing (FB) were planned using three techniques: wide tangents (WT) in FB with a standard match-plane, WT in FB with a superior match-plane and WT in DIBH with a standard match-plane. The standard match-plane was placed at the inferior aspect of the medial head of the clavicle. The superior match-plane was placed one CT slice (3 mm) below inferior aspect of the head of the humerus. Target volumes included the right axillary lymph node levels 1-4, whole breast and IMC (delineated according to ESTRO consensus guidelines). Ipsilateral lung $_{V17\ Gy}$, IMC and axillary nodal target volume dose statistics were compared between groups using a one-way ANOVA with Tukey's correction for multiple comparisons (significance level = 0.05).
Results: There was no significant difference between target volume coverage for the three techniques. The comparisons for mean right lung $V_{17\ Gy}$ for the three techniques are summarised in Table 1.

	WT(FB) Standard match-plane	WT(FB) Superior match-plane	WT(DIBH) Standard match-plane
Mean right lung $V_{17\ Gy}$ (%)	37.8	31.0	28.9
95% Confidence interval (%)	33.7–42.0	27.4–34.6	25.0–32.8

	WT(FB) Standard match-plane	WT(FB) Superior match-plane	WT(DIBH) Standard match-plane
Proportion of patients meeting the ipsilateral lung $V_{17\ Gy}$ constraint ($\leq 35\%$)	3/10	8/10	10/10

There was a statistically significant difference in right lung $V_{17\ Gy}$ between WT(FB) standard match-plane and WT(FB) superior match-plane ($p = 0.0004$). There was also a statistically significant difference between WT(FB) standard match-plane and WT(DIBH) standard match-plane ($p = 0.0016$). There was no significant difference between WT(FB) superior match-plane and WT(DIBH) standard match-plane ($p = 0.3059$)

Conclusion: DIBH is the most reliable technique for meeting the lung constraint in right breast-affected patients undergoing IMC-RT but cranial displacement of the match-plane offers a viable alternative where DIBH is not available.

P3.13

Is axillary dissection necessary after primary chemotherapy (pc) for node-positive breast cancer?

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Introduction: A full axillary dissection has been generally recommended for breast cancer patients who were node positive prior to PC. However, with excellent response rates in the breast, a more conservative approach in the axilla is suggested. We have, therefore, reviewed the axillary response rate in previously node-positive patients.
Method: We retrospectively audited the axillary response rate of biopsy-proven node-positive patients who underwent PC and axillary clearance over a 3-year period. We assessed the total number of nodes and the number of positive nodes per patient and the number who was node negative or who had only a single positive node. We also assessed radiological response rates in the breast and axilla.
Results: Thirty-eight node-positive patients were identified (mean age 54.8 years). Mean nodes obtained per patient were 14.7 (6–24) and positive nodes 3.1 (0–14). Ten patients had no residual positive nodes and a further ten had only a single positive node. Fifteen of these 20 patients had a partial or complete radiological response in the breast and 17 in the axilla.
Conclusion: In our series, over 50% of patients underwent "unnecessary" axillary clearance. We now select patients for post-chemotherapy sentinel node and sample based on the radiological response rate in the breast and axilla.

P3.14

Can surgical morbidity be reduced by primary chemotherapy (PC) down-staging of the initially positive axilla?

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Introduction: We have previously shown that PC converts over 50% of node-positive patients to node negativity or single positive node. In 2013, we switched to a selective policy whereby radiological assessments directed post-chemotherapy surgery. We reviewed the results to assess the practicality and safety of this policy.
Method: Biopsy-proven node-positive patients were assessed. Patients with one node had a coil insertion and selected for post-chemotherapy sentinel node/coiled node/sample (SCNS), whereas patients with two or more nodes underwent an axillary dissection. We assessed the total number of nodes and positive nodes in each group and compared this to our previous series who all underwent an axillary clearance.
Results: 36 node-positive patients were identified. Eighteen were selected for SCNS (mean 3.2 nodes harvested per patient) (2–7), whereas 18 had a full clearance (13.7 nodes, range 8–43). Eleven of the SCNS patients were node negative, and the other seven had only a single positive node. In the entire group (36 patients), the positive nodes harvested a mean of 2.5 nodes per patient (0–29) which was very similar to the harvest in our previous series where all patients underwent clearance.
Conclusion: By careful selection, 50% of patients avoided a full clearance. The total number of positive nodes remained unchanged (in comparison to a previous series with uniform full clearance) suggesting that undetected positive nodes are not being left in situ by this selective policy.

P3.15

Patient and surgeon satisfaction for cosmetic outcome with immediate implant-based breast reconstruction using titanium-coated polypropylene mesh

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Background: Titanium-coated polypropylene mesh (TCPM) is an alternative to acellular dermal matrix for the implant-based breast reconstruction. The primary aim of this study was to assess patients and surgeon satisfaction for cosmetic outcome after the procedure.
Methods: Between 2013 and 2017, implant breast reconstructions after conservative mastectomies using TCMP were performed in 37 patients (with 44 reconstructions). Patients were administered a short questionnaire (adapted from Breast Q) and surgeons graded the cosmetic outcome as excellent, good, fair or failed.

Results: Forty-four mastectomies with reconstructions were performed in 37 women. Twenty-eight patients had therapeutic mastectomies with nine of them also had contralateral risk-reducing mastectomies. Patient satisfaction survey was available in 27 patients. Overall, 91% of patients were satisfied with their cosmetic outcome when clothed and 65% were satisfied with their cosmetic outcome when unclothed. 25/27 patients thought that having reconstruction is a much better option. Surgeon's opinion was excellent in three, good in 29 and fair in four patients and failed in five patients. With median follow-up of 17.5 months, five of 37 patients had implant loss.
Conclusions: Most of the patients are satisfied by the cosmetic outcome after undergoing immediate breast reconstruction using the titanium-coated polypropylene mesh.

P3.16

Is there a role for microdochectomy in diagnosing early breast cancer in the modern era?

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Background: Nipple discharge is a common presentation of breast disease. Unilateral, single duct, blood-stained/serous/clear nipple discharge is a red flag symptom of breast cancer. Microdochectomy (excision of the offending duct) is used to provide a definitive histological diagnosis and symptom control.
Materials and methods: This is a retrospective analysis of prospectively maintained data. The data of patients who underwent microdochectomy between 1/1/2003 and 31/7/2017 were retrieved, entered onto an Excel database and analysed. Additional information, if required, was obtained from computerized records. The procedures were performed under the care of a single consultant surgeon.
Results: One hundred and four patients underwent 106 microdochectomies. Bloody/Brown/Black discharge was reported in 67(64%) of cases, 39(36%) had serous/clear discharge. 89(86%) of cases were benign. Of these, benign intraductal papilloma was reported in 39 cases (44%), Atypical Ductal Hyperplasia/Atypical Intra-Epithelial Proliferation-ADH/AIEP in 2 cases (2%), while Duct Ectasia and other benign breast conditions were reported in 48 cases (54%). 15(14.2%) patients had ductal carcinoma in situ (DCIS) on final histology. One case had frank invasion and another microinvasion. 13/15 patients with cancer had presented with bloody/brown/black discharge, but benign radiology (M2/U2). 7/15 patients had insufficient/normal cytology (C1/C2), except one patient who had a B3 core biopsy result.
Conclusion: A significant proportion of patients undergoing microdochectomy (14.2%) were diagnosed with breast cancer. All those patients had normal or benign radiology reported prior to surgery. There was no significant correlation between a final diagnosis of cancer with cytology of nipple discharge, colour of discharge and dipstick positivity for blood.
Microdochectomy remains a useful diagnostic tool in patients presenting with unilateral single duct nipple discharge in the absence of other breast symptoms and normal/benign imaging. These cases would not have been diagnosed, but for this procedure. It also provides symptomatic relief in up to 99% of patients.

P3.17

Perioperative blood transfusion requirements following elective major breast cancer surgery

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Aim: Blood loss during contemporary breast cancer surgery is limited. The routine request for Group and Save (G&S) is likely unnecessary for the majority of patients undergoing this type of surgery. We reviewed the records of all patients undergoing major elective breast cancer surgery to identify the frequency of blood transfusion.
Methods: All consecutive patients undergoing mastectomy and/or axillary node clearance in a single Scottish health board from January 2012 to July 2016 were included. Electronic health records and transfusion laboratory database were reviewed to establish pre- and post-operative haematological indices, G&S request, blood crossmatch request and blood transfusion prescriptions.
Results: In total, 531 patients underwent mastectomy and/or axillary node clearance. Of these, 486 (91.5%) had a recorded G&S request. In total, 15 (2.8%) patients had a blood crossmatch request performed and 9 (1.7%) received a blood transfusion. One patient received the blood transfusion pre-operatively for anaemia. No patient required blood transfusion on the day of surgery for post-operative bleeding. Of the seven patients who experienced a post-operative bleed requiring transfusion, only one patient was taking antiplatelet medication.
Conclusions: This audit showed that blood transfusion requirement following elective major breast surgery is very low and is rarely required as an emergency. Routine requesting of G&S is not required prior to this kind of surgery.

P3.18

Magnetic seeds: an attractive option for localisation of impalpable breast cancers

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Introduction: Accurate localisation of impalpable breast lesions is essential to optimise both oncological and non-oncological outcomes for breast-conserving surgery. A number of techniques are employed to achieve this, the most common in the UK being wire-guided localisation. Wire placement adds stress for the patients on the day of surgery and can cause delays to the operating schedule. Radioactive Seed Localisation (RSL) is another method; however, this poses specific logistical difficulties associated with radioisotope legislation. Magnetic seed localisation (Magseed) has similar principles to RSL. It can avoid a same-day placement, and operative time may be reduced without compromising accuracy.
Methods: A pilot study of ten patients has been undertaken to investigate Magseed for localisation and removal of impalpable breast lesions in breast-conserving surgery. Surgeon and radiologist satisfaction with the technique was assessed.
Results: In nine out of 10 cases, the seed was implanted within 5 mm of the target lesion and one seed was placed 6 mm from the target lesion. The radiologists found the Magseed easy to insert (median score 5/5). Eight were inserted under ultrasound and two under

stereotactic guidance. Nine patients have undergone surgery, all with successful removal of the seed and the surgeons were satisfied with the technique (median score 5/5). Nine patients have surgical pathology results available of whom one had a positive margin (11%), but the sample size is too small to draw any conclusions on this metric.

Conclusions: The Magseed localisation technique can be performed safely and easily as an alternative to wire localisation. We have already seen tangible benefits to both our clinical team (in terms of flexibility of scheduling) and, most importantly, the patients, but in depth study is warranted to quantify the impact from patient, clinician and financial perspectives.

P3.19

Margin width and local recurrence after breast conserving surgery for ductal carcinoma in situ (DCIS)

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Background: Ductal carcinoma in situ (DCIS) represents 5% of symptomatic and 20–30% of screen detected cancers. Breast conserving surgery (BCS) ± radiotherapy (RT) is performed in over 70% of women with DCIS. A recent US consensus suggested that a 2-mm margin was optimal but this was in part because there were no reliable data that had compared 1 mm with 2-mm margins in DCIS. This study provides that data.

Methods: A single institution study of 466 patients (median age 61, range 35–94) with DCIS treated by BCS between 2000 and 2010 was carried out. Clinical characteristics are summarised in Table 1. 292 (63%) received whole breast RT and 174 (37%) did not. Patients were selected for RT based on perceived risk of in breast tumour recurrence (IBTR). RT was advised for all patients with high-grade DCIS after 2003 and before this RT was used selectively based on extent and grade of DCIS. Distance to nearest margin was measured to 0.1 mm; ten patients had margin width < 1 mm, 94 widths of 1–2 mm and 362 widths of > 2 mm. There was no association of margin width with use of RT.

Results: At a median follow up of 7.2 years, there were 44 IBTR (27 DCIS and 17 invasive). The 5-year IBTR rate fell significantly from 2000–2005 to 2006–2010 with a hazard ratio of 0.456 ($p = 0.021$). For margin widths of 1–2 mm actuarial IBTR rate was 8% at 5 years, and 10% at 10 years; for margin widths of > 2 mm the IBTR rate was 8% at 5 years, and 13% at 10 years. There was no evidence that patients with margins of > 2 mm had a lower recurrence rate than patients with margins of 1–2 mm. Odds Ratio for IBTR 1–2 mm versus > 2 mm was 0.776 (95% CI 0.333–1.811) $p = 0.558$. There were too few patients with margins of < 1 mm to include in the analysis. There was no association between margin width and histological grade, comedo necrosis, age or DCIS size. In a multivariate analysis, only DCIS size predicted for IBTR (HR 2.73 $p < 0.0001$). 292 patients received whole breast RT. Patients who received RT were more likely to have high-grade DCIS, comedo necrosis, large areas of DCIS ($p < 0.0001$). There was no significant difference in IBTR rate between the patients who did and who did not receive RT ($p = 0.777$). Rates of IBTR for the 167 patients in the no RT group versus the 292 who got RT were 1.8 versus 2.74% invasive and 3.59 versus 5.14% DCIS at 5 years and 2.99 versus 4.11% invasive versus and 7.1 versus 5.14% DCIS at 10 years. No patients in this series died from breast cancer.

Discussion:

- These new data show that, as in invasive breast cancer, 1 mm is a sufficient margin width for BCS in DCIS, irrespective of whether patients have RT.
- Selective use of RT reduces the rate of IBTR in high-risk DCIS lesions to a rate similar to those of lower risk lesions.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

Table 1 Clinical characteristics of patients

Characteristic	Number of patients
DCIS size	
< 20 mm	269 (58%)
20–40 mm	179 (38%)
> 40 mm	18 (4%)
Grade	
1	35 (7%)
2	120 (26%)
3	311 (67%)

P3.20

Long-term outcome of neoadjuvant endocrine therapy followed by breast conserving surgery

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Background: Neoadjuvant therapy (NET) in women with large or locally advanced estrogen receptor(ER)-rich breast cancers (BC) allows the option of breast-conserving surgery (BCS)+ radiotherapy (RT). The aim was to study the long-term safety of this strategy.

Methods: Two hundred and eighty postmenopausal women (median age 77, range 50 to 95—see Table 1) with ER-rich BC had BCS after NET (median duration 4.8 months, range 1.7–42.8 months). 221 (79%) received only letrozole. 59 (21%) began on letrozole and were switched to anastrozole, exemestane or tamoxifen due to adverse events or lack of clinical response. Two hundred patients (71%) received adjuvant RT (RTgroup) and 25 (9%) received adjuvant chemotherapy. Median follow-up was 5.5 years.

Results: 254 patients had NET response data. 74% had a clinical response. NET response was higher for grade 1 (84%) than for grade 2 (73%) or 3 (72%) cancers.

Actuarial local recurrence rates (LRR) were 8% (95% CI ± 0.04) and 12% (95% CI ± 0.06) at 5 and 10 years. Actuarial overall BC recurrence rates were 14% (95% CI ± 0.04) and 27% (95% CI ± 0.12) at 5 and 10 years, with BCS death rates of 7% (95% CI ± 0.04) and 14% (95% CI ± 0.10) at 5 and 10 years, showing only half with recurrence died from BC. Crude all-cause mortality but not BC-specific survival (BCSS) favoured those who had adjuvant RT

($p < 0.001$) or chemotherapy ($p = 0.006$). The 15-year rate was 50.9%, while BCS death rate was only 7.3%.

Positive nodes were associated with worse overall recurrence-free survival (RFS) ($p = 0.007$) but not local RFS or BCSS. Tumour size was not associated with RFS or BCSS. Tumour grade was not associated with RFS but grade 3 patients had a lower BCSS ($p = 0.002$) compared to patients grade 1/2 cancers. RT was associated with improved LRR ($p < 0.0001$) and overall RR ($p = 0.038$): 5 and 10 years in RTgroup were 5% (95% CI ± 0.04) + 7% (95% CI ± 0.04) versus 9% (95% CI ± 0.12) + 31% (95% CI ± 0.24) in no-RTgroup. The 5- and 10-year ORR in the RTgroup were 14% (95% CI ± 0.06) and 39% (95% CI ± 0.16) versus 28% (95% CI ± 0.16) + 38% (95% CI ± 0.24) in the no-RTgroup. Although differences were not significant, BCSS was higher in the no-RTgroup: 5 and 10 yearly BCSS rates were 10% (95% CI ± 0.04) and 20% (95% CI ± 0.12) versus 6% (95% CI ± 0.08) + 12% (95% CI ± 0.14) in RTgroup.

16/67 patients with T3/4 cancers who did not receive RT had lower overall RFS ($p = 0.018$) but no difference in local RFS. 13/98 patients with node-positive disease whom did not receive RT had lower LRR ($p = 0.002$) and overall RR ($p = 0.024$). 54/169 node-negative patients whom did not receive RT had lower LRR ($p = 0.019$) but similar overall RR. 50/183 patients with grade 1/2 cancers did not have RT and had lower LRR ($p < 0.0001$) and overall RR ($p = 0.049$). BCSS was not associated with RT use in subgroups related to tumour size, node status or grade.

Discussion:

- Response to NET is not worse in ER-rich grade 3 or node-positive cancers.
- After NET, BCS and RT provide excellent LRR.
- BCSS rates were low; most died of other causes.
- NET followed by BCS and RT is safe even for grade 3 and node-positive cancers.
- BCS alone provides adequate disease control for majority with significant co-morbidities

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

Table 1 Clinical characteristics of patients

Characteristic	Number of patients
Tumour size	
T1	31 (11%)
T2	169 (60%)
T3	14 (5%)
T4	56 (20%)
Unknown	10 (4%)
Grade	
1	36 (13%)
2	153 (55%)
3	70 (25%)
Unknown	21 (7%)
Nodes	
Positive	101 (36%)
Negative	177 (63%)
Unknown	3 (1%)
ER Allred score	
6	15 (5%)
7	62 (22%)
8	203 (73%)

P3.21

Short-term outcomes of immediate breast reconstruction in a university teaching hospital

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Introduction: Risk assessment for breast reconstruction commencing at the diagnostic clinic regardless of subsequent reconstructive pathway/type can be under or overestimated subjectively. We performed retrospective objective risk assessment using previously published chart and present post-operative outcomes up to 3 months (consistent with NMBRA audit, UK, 2011) including deemed higher-risk variables in a university hospital.

Methods: Ninety reconstructive procedures performed over 21 months [March 2014–December 2015 (after exclusion of 27 risk-reducing mastectomies)] were studied. Pre-operative variables were correlated with complications using linear regression analysis (statistical program R3.3.3).

Results: Overall, 33 post-operative wound (breast or donor site in autologous) adverse outcomes that settled with outpatients' intervention including 23 uncomplicated seroma aspirations (18 donor site, four axillary, two breast) and seven delayed wound healing. Three were re-admitted to hospital (re-operation-2; IV antibiotics-1) and six needed re-operation (Irrigation-washout = 2, donor site bleeding = 1, inpatient and outpatients' wound debridement = 3). Apart from seroma, in the short-term, skin flap necrosis, capsular contracture, asymmetry, dog ear, implant revision, other breast issues (erythema/pain), lymphedema and other miscellaneous (contact dermatitis etc.) were less than 5%. Breast sizes E and larger and Grade 3 or higher ptosis correlated significantly with complications.

29 patients were pre-operatively certain to receive post-operative RT and out of these four had delayed healing, one needing debridement and one developed a DVT.

Conclusions: Surgical choices are mostly offered based on clinician's assessment of multiple factors followed by the expectation for the patient to arrive at a decision when already overwhelmed with cancer diagnosis. Local objective outcome data (especially in presence of factors that put satisfaction at risk) based on objective assessment may help the subjective shared-decision making especially when the outcome data are local as opposed to generalised population/published data. Outcomes prediction is limited by small numbers and may be answered by upcoming national databases.

P3.22

Effect of sleeve application for lymphoedema on quality of life (QoL) and arm volume swelling (results of prospective BEA study)

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Application of an external compression sleeve is recommended to treat lymphoedema (defined by Relative Arm Volume Increase (RAVI) > 10%). In a prospective NIHR study (UKCRN ID 8881), 1100 patients undergoing axillary clearance had pre-operative and post-surgery arm measurements by perometry, Bioimpedance (BIS (L-Dex)) and provided, FACT-B + 4 and lymphoedema checklist questionnaires. RAVI of > 10% diagnosed lymphoedema. By 24 months after surgery 219 (21.1%) patients required sleeve application.

To determine patients benefiting from sleeve application, RAVI, QoL and self-reported changes were related to effects of treatment on QoL.

	n	Estimated marginal mean (95% CI)				*p* value
		Pre surgery	Before sleeve applied	At sleeve application	At 36 months	
Total Fact-B+ 4	214	106.9 (104.1–109.7)	103.6 (100.5–106.5)	105.0 (102.0–107.9)	108.5 (104.8–111.9)	0.001
ARM	212	18.7 (18.3–19.0)	15.4 (14.8–15.9)	14.2 (13.6–14.8)	15.2 (14.4–15.8)	< 0.001
TOI	214	66.7 (64.7–68.6)	62.7 (60.6–64.7)	63.6 (61.5–65.7)	66.8 (64.3–69.3)	< 0.001

After treatment with a sleeve, an increase in QoL occurred (see table).

Patients with self-reported arm swelling (B3) scores (range 0–4) separated into a group with considerable swelling (58%)—with larger RAVI values compared to a group with little to no self-reported arm swelling (42%) at the time of sleeve application ($p < 0.001$, median 8.40 vs 4.55%). Overall, QoL scores for patients with little/no swelling were higher than those with considerable self-reported arm swelling at sleeve application and (FACT-B+ 4: $p = 0.001$, TOI $p < 0.001$) at 36-month post-surgery.

In patients with considerable swelling (but not little/no swelling), mean FACT-B and TOI (QoL) increased following sleeve application by 48 months of post-surgery (FACT B+ 4: $p = 0.024$, TOI: $p < 0.001$). The pattern of change over time differed between those with considerable swelling versus no/little swelling in the ARM question ($p = 0.005$).

Prescription of Compression sleeves in "correctly diagnosed" lymphoedema (RAVI > 5% and considerable self-reported swelling) improves symptoms and QoL. This has important implications for Compression sleeve prescription in the NHS.

P3.23

Is it time to relook the management of axilla in post-neoadjuvant breast cancer cases in a re-evolving era of current axillary management?

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Axillary Node Dissection (AND) is an established practice for more than a century for its role in staging and adjuvant treatment decisions, as are its accompanying serious complications. In spite of land mark, Z0011 and AMAROS trials, we are reluctant to pass this benefit to Neoadjuvant cases, although the above cited reasons of AND are changing too.

Methods: A prospective study of 100 cases of neoadjuvant chemotherapy.

Results: Sixty-six patients have axillary core-positive nodes which after NAC had AND, 25 (38%) showed complete pathological (pCR) response in axillary nodes, while 41 (62%) have residual disease. Among the 25 patients with complete axillary response, 17 have pCR in the breast too, and of these, 9/17 would have been eligible for Z0011 criteria without NAC. While 34 have normal axillary US or core-negative nodes, five had upfront SLNB and were excluded from analysis. The remaining 29 cases had SLNB after NAC with 24 (83%) node negative, 4 (14%) positive and one showing pCR with fibrosis in two SLNB nodes; these five went on to have AND. The SLNB positive rate was lower as compared to a similar cohort of cases without NAC, although former has significantly worst prognostic profile, which highlights the downstaging impact of NAC. In this group, 8 patients have pCR both in breast and axilla, 5/8 were eligible for Z0011 criteria without NAC.

These results provide a convincing argument to avoid AND in complete responders; a similar wish is expressed by the Dutch survey.

Conclusion: Therefore, we conclude that it is time to relook and produce new guideline for axillary management in post NAC cases with pCR and possibly avoiding AND in nearly 30–40% core-positive axillary nodes.

P3.24

Is Lipomodelling a Safe Procedure for Breast Reconstruction, Single Surgeon Audit of Practice?

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Introduction: There is an increased demand by breast cancer patients as well as those with congenital and acquired defects for reconstructive and corrective surgery. There are high expectations of patients for good aesthetic outcome. Lipomodelling is increasingly used as a recognised procedure for breast reconstruction and corrective surgery. Common indications include Breast cancer surgery, developmental defects and aesthetic reasons.

This audit of practice of a single surgeon was performed using ABS and BAPRAS (2012) guidelines.

Methods: This single center audit was performed prospectively. Data were collected and assessed for all patients undergoing lipomodelling for 18 months.

Results: Forty patients underwent a total of 53 sessions. Eleven patients (27.5%) underwent two sessions and 2 (5%) had three sessions. Out of 40 patients, 33 (82.5%) had cancer surgery, 3 (7.5%) cosmetic and 4 (10%) Developmental indications. The age range was between 20 and 74 years, with a mean age for cancer patients was 52.45 (34–74), cosmetic 43 (30–66), and ford developmental abnormalities was 21.75 (20–24) years.

Forty-seven (89%) patients underwent lipomodelling using Coleman technique, and for 6 (11%) patients PURE graft technique was used. Most common site for fat harvesting was the abdomen followed by sides and back. For many patients, fat was harvested from a combination of sites.

Average amount of fat harvested was 257 mls and average fat grafted 146 mls. Hence, the percentage of fat removed that is grafted—56.5%

Conclusions: Lipomodelling is a safe procedure for breast reconstruction, which can be done as a daycase. Patients tolerate the procedure and accept more than one session for lipomodelling.

DIAGNOSTICS—IMAGING

P4.1

Does mammographic density in women with previous breast cancer predict further breast cancer?

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Background: The aim of this research was to determine whether increased mammographic density in women with a previously diagnosed breast cancer is related to the risk of development or detection of a further breast cancer in either breast.
Methods: Nine hundred and six women with previous breast cancer were identified from those consenting to the Predicting Risk of Cancer At Screening (PROCAS) study. Personal risk factors were self-reported, with visual assessment of mammographic density recorded by two readers on Visual Analogue Scales (VAS) and automated volumetric breast density measured using Volpara™. Of these women, 23 had a subsequent contralateral breast cancer and 17 had subsequent ipsilateral breast cancer. A nested case control study was carried out where cases were women with a second breast cancer. Three controls per case were matched on age, HRT use, menopausal status, parity and BMI.
Results: Increased dense volume in the breast contralateral to that of the previous cancer was associated with subsequent development of cancer in that breast, with median fibroglandular volumes of 44.8 and 37.9 cm^3, respectively, ($p < 0.05$) for cases and controls, although no significant difference between cases and controls was found for volumetric or visually assessed percent density. No association between increased mammographic density and a second ipsilateral breast cancer was found.
Discussion: Our results suggest that increased fibroglandular volume might be predictive of subsequent contralateral breast cancers in women with a previous diagnosis of breast cancer, but further investigation with a larger sample size is needed to confirm this result.

P4.2

Understanding breast density—insights from high resolution X-ray imaging

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Despite earlier diagnosis and more effective therapies, breast cancer still affects one in 8 women in the UK. Low-dose radiography of the breast (mammography) is the main diagnostic tool and raised mammographic density (MD) is a strong risk factor for the development of breast cancer. Until recently, it was assumed that increased MD was associated with novel collagen deposition; however, our published work has shown that, in post-menopausal women, MD is associated with the reorganisation of collagen into large diameter, mechanically stiff bundles—an established risk factor for cancer in a number of tissues.

To investigate the influence of global MD on local tissue structure and X-ray density, we recruited post-menopausal women (57–63 years) undergoing mastectomy for invasive breast cancer at the University Hospital of South Manchester. Both tumour and non-tumour tissue samples (located at least 4 cm from the tumour) were collected from the same breast before being fixed and wax embedded and these blocks were used for subsequent X-ray imaging by laboratory and synchrotron X-ray sources.

Using laboratory phase contrast micro-computerised tomography (uCT), we were able to resolve key biological structures including stromal bundles, epithelia and adipose tissue. We also observed multiple, punctate regions of high X-ray attenuation measuring 1-10 micrometres. These presumptive micro-calcifications are at least two orders of magnitude smaller than the mm-scale micro-calcifications, which are associated with increased breast cancer risk in mammograms.

Contrast (and therefore MD) in clinical mammograms is imparted by differential X-ray attenuation rather than phase shift. In order to understand the influence of tissue micro-structure on MD, we used a multimodal technique known as speckle imaging generating both phase and absorption information at the B16 beamline of the Diamond Light Source synchrotron to map the 3D X-ray/tissue electron density at μm length scales.

Analysis of this X-ray imaging data alongside genomic, proteomic, and micro-mechanical data will allow us to interrogate the role of local tissue structure in MD and inform future therapies which could reduce breast density and cancer risk.

P4.3

Do patients with breast cancer and positive axillary core biopsy (CB) have a higher burden of axillary disease than those with negative preoperative assessment?

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Aims: To compare the burden of axillary lymph nodes (LNs) disease in breast cancer patients with no proven metastases pre-operatively versus core biopsy proven LN metastases.
Methods: Of 1457 breast cancer patients treated in NHS Tayside over a 3-year period, 260 had positive nodal metastases excised at sentinel node biopsy (SNB) or ANC. Data including imaging and histopathology of CB and LN positivity were retrospectively reviewed from local cancer audit database.
Results: Of 260 patients with positive LNs, 113 had ANC as first axillary procedure for metastases shown on CB (**Group A**), while 80 patients had positive SNB after normal USS or normal CB (**Group B**). Of **Group B**, 26 proceeded to ANC, while 54 had axillary radiotherapy. There was a significant difference in the median number of abnormal LNs involved in Group A versus Group B (4.0 vs. 1.0; $p < 0.0001$), as more LNs in total were excised with ANC. When comparing only those who had ANC in Group A versus Group B, there was a significant difference in median number of positive nodes (4.0 vs. 2.0; $p = 0.0485$). Of those who had ANC, 57 (41%) had only 1 or 2 nodes positive. Comparing all ANC procedures for factors predicting 1–2 nodes positive versus more than two nodes positive,

USS did not predict for heavier node positivity ($p = 0.14$), but referral through breast screening ($p = 0.004$), smaller tumour size (23.8 mm V 41.0; $p < 0.0001$) and ER+ Her2− tumours ($p = 0.04$) all showed statistical significance for fewer positive nodes.
Conclusion: In patients with CB proven metastases, there is a higher disease burden than those who have normal USS or CB pre-operatively. 41% of patients having ANC have only 1–2 nodes positive. Our data suggest a subgroup of patients in whom ANC may be avoided even in the presence of CB proven metastases pre-operatively.

P4.4

The overestimation and the inappropriate promotion of the benefits of mammographic screening in the research and interventions into breast cancer in Gaza

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Background: There has been extensive debate about whether mammographic screening has done more good than harm. Recent reviews found that women who undergo mammographic screening are more likely to have breast cancer that was an over-diagnosis, and therefore would not cause them problems. In Gaza, there is a strong forum of people who advocate an increase in mammographic screening in Gaza, and the aim of this review was to establish the evidence for this view.
Methods: Research papers that have focused on breast screening in Gaza (or elsewhere in Palestine) were identified. Published educational material including pamphlets, booklets, and short videos on breast screening were also reviewed. A thematic analysis was carried out to document the opinions and citations of authors on the effect of mammography.
Findings: Eleven studies were identified, 7 cross-sectional studies, and 4 retrospective cohort studies. Twenty educational materials were located. Six cross-sectional studies found that 60–80% of Palestinian women had never had a mammogram, while one study found that 90% of women in Gaza were willing to undergo diagnostic mammography but less than 30% were willing to undergo mammographic screening. Some of these studies argued that mammographic screening programs would improve survival by more than 20% in Gaza. Two retrospective cohort studies concluded that Palestinian women have low survival rates due to the poor availability of mammography, and another could not measure the association between mammography and women's morbidity due to the lack of complete data at the cancer registry. Only three mentioned possible harmful effects of mammographic screening. All educational materials had clear understandable information on the benefits of breast cancer mammographic screening, but minimal information on its harms.
Interpretation: Research in Gaza has overestimated the benefits of mammographic screening, and this would encourage women to undergo screening without knowing that it could harm them. More effort should ensure appropriate surgery, radiotherapy and chemotherapy for women with breast cancer symptoms.
Acknowledgments

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P4.5

Changing upgrade rates for B3 lesions following introduction of first-line 10G-vacuum assisted biopsy

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In 2012, we introduced first-line 10G-vacuum-assisted biopsy (VAB) for all stereotactic biopsies. Prior to this, we retrospectively evaluated the upgrade rate to malignancy of B3 lesions diagnosed within the unit, following an initial 14G-biopsy. Following the introduction of 10G-VAB, all B3 lesions identified have been entered into a prospective database and outcomes of second-line 7G-VAB or excision biopsy recorded.

Comparison of outcomes between retrospective audit from 09/2009 to 08/2011 of all B3 lesions identified following initial 14G-core biopsy and prospective audit of all B3 lesions identified on 10G-VAB or ultrasound-guided 14G-biopsy between 01/2012 and 12/2016.

Between 2009 and 2011, 171 B3 lesions were identified over 2 years. 32/171 lesions were upgraded at this time, giving an overall 18% malignancy rate. Between 2012 and 2016, 467 B3 lesions were identified, with 55 upgraded, giving an 11% overall malignancy rate. Considering only those B3 lesions identified by initial 10G-VAB, upgrade rate was 19/251 (7.5%). In absolute numbers, over 5 years, the number of B3 lesions identified per year has decreased from 111 to 67. Average weight for 10G-VAB samples was 2.54 g (range 0.56–11.3 g).

A reduction in B3-to-malignancy upgrade rates was observed following implementation of first-line, stereotactic 10G-VAB, despite consistent cancer detection rates. In addition, absolute numbers of B3 lesions appear to have reduced since implementation. This implies improved cancer detection with first-line 10G-VAB biopsy, due to the increased sample sizes provided using a 10G compared to 14G device. Guidelines for B3 lesion management may have to consider different management protocols depending on the first-line devices used.

P4.6

Prospective follow up of patients with B3 lesions over a 4-year period

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Following introduction of large-bore vacuum-assisted biopsy (LVB) for diagnosis and management of B3 lesions in 2011, a prospective database of patients was developed. Following B3 lesion diagnosis on core biopsy, patients underwent LVB. If B5, patients had surgery; if B3 patients underwent 5 years annual surveillance mammography (ASM) or were discharged/returned to routine recall, depending on the presence of epithelial atypia. Outcomes were prospectively audited over 4 years.

B3 lesion database analysed to ascertain number of ASMs performed, recall rate, symptomatic episodes and subsequent malignancy following B3 diagnosis.

Between October 2011 and December 2015, 396 patients had a B3 lesion. Three hundred and five underwent second line LVB. Twenty-seven patients diagnosed with malignancy following LVB and 17

patients were upgraded to malignancy following excision biopsy (unsuitable LVB/pathology request).

Three hundred and fifty-two patients had ASM/routine recall, together having 410 mammograms performed over 4 years. Nine patients recalled from ASM (recall rate 2%). Twenty underwent further breast investigations (19 presented symptomatically, one recalled from MRI high-risk surveillance).

From additional investigations, three cancers diagnosed (one following high-risk surveillance MRI; one symptomatic presentation, one recall from ASM). There were also four further B3 lesions and 22 benign diagnoses.

LVB for B3 lesions is an excellent alternative to excision biopsy. However, ASM has a low recall rate and cancer detection rate, with only one of the 3 subsequent cancers detected by mammographic surveillance. This questions whether ASM is really necessary in this group of patients. Could they be safely returned to routine recall within the screening program?

EPIDEMIOLOGY AND PREVENTION

P5.1

A mini-review of oral contraceptives to evaluate and define their contribution to breast cancer risk prediction

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Background: Breast cancer is the most common cancer among women in England. Oral contraceptives (OCs), one of the most popular forms of contraception in the United Kingdom, have been implicated in causing breast cancer.
Aims: We evaluated and defined the contribution of OCs to breast cancer risk, and whether this differed with type and duration. We also addressed how this information could be utilised for the development and validation of the CanRisk cancer risk assessment tool.
Method: We conducted a systematic review following the methodology recommended in the PRISMA Group (2009) [1].
Results and discussion: Of the 2079 unique citations screened, seven studies relevant to breast cancer in the UK were identified. The risk of 'Ever-user' of OCs on breast cancer incidence was modest, and typically found in 'current' and 'recent' OC users. This effect was lost within 10 years, and the association was largely independent of duration or type. These findings allowed us to develop the CanRisk tool and pose two questions related to usage and most recent use.
Conclusion: The actual relationship between 'Ever-' versus 'Never-' users was far more complex, with positive associations found between use and breast cancer risk in 'current' and 'recent' users, young women, and before first-full term birth. Whether these relationships were all interconnected was beyond the scope of this analysis but remain points of interest requiring further investigation.

P5.2

Reduction in breast density after 5 years of tamoxifen estimated by automated techniques: The use of automated methods of breast density estimation to determine response to tamoxifen at 1 and 5 years of preventive therapy

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Background: Previous studies have demonstrated that treatment with tamoxifen reduces breast density in a proportion of women. This is important since density reduction assessed visually is correlated with the long-term preventative effect of tamoxifen (Cuzick J et al. JNCI 2011). Here, we investigate the value of automated techniques for estimating density reduction using estimates of change in dense volume (Volpara, V), dense area (Densitas, D) and in a measure of BIRADs (Stratus,S) in comparison with visual assessment as percent density (PD).
Methods: Women at increased risk of breast cancer ($n = 135$) aged 33–46 agreed to take tamoxifen for 5 years for breast cancer prevention. All were undergoing annual mammography as part of an early detection programme. Controls ($n = 204$) were of the same age and risk undergoing annual mammography in the same clinic.
In our previous study, women with visually assessed density below the median of 10% did not develop breast cancer; we report response data for each technique related to median change.
Results: Median density in women taking tamoxifen at baseline and 1 year for V, D and S were 61.3–50 ccs, 43.5–35.0 cm^2 to and 3.5–3.0 BIRADs units, respectively. There was no significant reduction in controls. The median and interquartile ranges for change in density were 10.9 cc (2.7–21.1), 6cm^2 (3.0–11.0) and 0.3 units (0.0–0.4), respectively. The median of the change in percent density (PD) was 10% (5.0–15.0). Correlations of change between techniques for all women at 1 year were PDvV (0.26), PDvD (0.14), PDvS (0.43), VvD (0.52), VvS (0.28) and DvS (0.08). Fifty-seven women stopped tamoxifen at or before 1 year. Of these, 22 (38.6%) had a marked increase in subsequent density measures. Sixty-nine women have continued tamoxifen for 4–5 years to date: 29/69 (42.0%) have had sustained reductions in density below the medians by all three automated techniques. In 30/69 (58.0%), there was no response by one or two methods, and in 10/69 (14.5%) there was no response by any technique.
Discussion: This study confirms that tamoxifen produces marked reductions in density measured by automated techniques based on change in dense volume, dense area and BIRADs which can be used to assess response to tamoxifen in women on an annual mammography

programme. However, correlations between techniques were relatively low and suggests that more than one technique might be used in practice. Virtually, all of the reduction in density seen at 1 year was sustained for as long as treatment continued. Whether the marked rise in density after cessation of tamoxifen seen in some women is detrimental remains to be investigated. In summary, we demonstrate that change in density as a result of tamoxifen treatment may be evaluated by automated techniques which may be more applicable than visual techniques in the clinic.
This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P5.3

Incidence of other previously diagnosed primary malignant tumor types in breast cancer patients; a retrospective statistical evaluation in 2007–2016

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Second primary malignancies in breast cancer patients are becoming an issue of concern worldwide. Using clinical records from the Dept. of Clinical Pathology and Genetics, we performed a retrospective search for other types of previously diagnosed primary malignancies in 6717 breast cancer patients registered with newly diagnosed primary invasive and/or in situ breast carcinomas during a 10-year period (2007–2016). The yearly incidence of breast cancer patients with other previous primary malignant tumors ($n = 332$ patients in 10-year period, including three male patients), increased from an average of 2.4–7.9% during this 10-year period and ranged from 16 to 57 patients. The most striking increase in incidence was found among the gynecological tumors (endometrium and ovarian adenocarcinomas), malignant melanoma and gastrointestinal malignancies. The overall survival rates for cancer patients have improved tremendously during the past 40 years, in part due to individually tailored therapies. As cancer patients live longer, they have elevated risk to develop primary malignancies later, in other organs.

Second malignancies that occur in long-term cancer survivors may be due to sporadic cancers that would have occurred anyway, where environmental, genetic, reproductive, and lifestyle factors (smoking, alcohol consumption, and body mass index) may play a role. Identifying the type of different coexisting primary malignancies may awake a special clinical vigilance for oncologists, warranting new screening programs for cancer patients to detect certain second or third, etc. primary malignancies at an early stage.

Patients with several primary malignancies need special treatment strategies, regarding the previously administered radio- and/or chemotherapy, to avoid excessive cytotoxic harm due to cumulative effect of all applied therapies.

P5.4

Breast cancer survival in Soweto, South Africa: a retrospective cohort study of women diagnosed 2009-11

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In Sub-Saharan Africa (SSA), breast cancer incidence is lower than in western countries but mortality is as high. South Africa's public healthcare system is better equipped to manage breast cancer than most other SSA countries, but the impact on survival is unknown.

A retrospective cohort of 602 women newly diagnosed with invasive breast carcinoma from 2009 to 2011 at Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg, was assembled. Survival analysis of time from diagnosis to time of death ('ideal' outcome) or terminal disease was performed. Losses to follow-up were also examined. Cox regression was used to estimate hazard ratios (HR) associated with woman and tumour characteristics.

During a median 2.1-year follow-up (IQR 0.5–3.8), 149 women died or were classified terminally ill; 287 were lost-to-follow-up. Overall survival differed significantly by stage at diagnosis (3-year survival: 84% early stage (I/II) versus 56% late stage(III/IV); HR 2.8 (95% CI 1.9–4.1)).

Losses to follow-up over the same period were 34% early stage and 51% late stage. Women remaining in contact with the healthcare system for at least 6 months after diagnosis showed better survival (84% early stage and 62% late stage) and had fewer losses to follow-up (21% early stage and 34% late stage). After mutual adjustment for stage, grade, age, intrinsic subtype and HIV status, lower survival was also associated with triple-negative [HR 3.1 (95% CI 1.9–5.0)] and HER2-enriched [2.5 (95% CI 1.4–4.5)] compared to ER/PR+ HER2− tumours. HIV-positive women did not have a significantly different risk for survival.

In this South African cohort, breast cancer survival was dependent on stage at diagnosis and tumour hormone receptor status. Early presentation and accelerated referral and diagnosis, coupled with treatment, are needed to prevent breast cancer deaths, and survival improvements need to be monitored using prospective studies with active follow-up.

P5.5

Attendance at breast screening—exploring potential reasons for lower uptake in areas of social deprivation

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Background: The Scottish Detect Cancer Early programme aims to improve overall 5-year survival for people diagnosed with cancer. A key aspect is improving informed consent and participation in national cancer screening programmes. NHS Lanarkshire includes some of the most deprived areas in Scotland and has a lower than average attendance at breast screening (68.4% uptake in North Lanarkshire compared with 73.5% national average). This study aims to explore attitudes towards and potential barriers to attending breast screening in our population.
Methods: All women over 50 years old attending symptomatic breast clinics were asked to complete a questionnaire. It explored potential reasons for not attending screening and asked questions such as access to screening or concerns about discomfort. The questions were compiled by clinicians in the symptomatic, screening and public health services.
Results: Following analysis of a pilot study where work commitments were a significant barrier to attendance at screening, 216 patients of screening age (50–70) were reviewed and all women over 50 surveyed. 56% of these were from the most deprived areas. In this study, only 10.8% of patients did not attend (DNA) breast screening but 67% of DNAs were from deprived areas. Reasons for this included forgetting appointments, work commitments, concern about outcome, hospital follow-up and machine failure at time of appointment. No one factor was a significant barrier and no patients described access or travelling as a problem.
Conclusion: In this survey of women of breast screening age attending symptomatic clinics, attendance at screening was better than the national rate. This would appear to be a more health motivated group. Of those patients who had missed appointments at screening, there were no consistent barriers to attendance but non-attendance was higher in deprived areas. Wider primary care surveys in low attendance areas would be a useful follow-up study.

P5.6

Treatment resistant breast cancer patients over four decades

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Introduction: Breast cancer is the most common cancer in the UK and the second cause of cancer deaths in women. Since the 1970s, there have been major advances in the diagnosis and treatment leading to a significant reduction in mortality. However, a sub-group of patients remain resistant to treatment strategies and die from the disease.
Material and methods: Between January 1st 1975 and December 31st 2006, a total of 5502 invasive breast cancer patients received surgery at Guy's NHS Trust Hospital, London, UK. Data on patient demographics, tumour characteristics, treatment, recurrence, metastasis and outcome were prospectively recorded. For analysis, the patients were divided into four time periods, i.e. 1975–1982, 1983–1990, 1991–1998 and 1999–2006, defined by the time periods in which they received their initial diagnosis. Time to event analysis was performed by means of Cox proportional hazards model and Kaplan–Meier estimation.
Results: The unadjusted hazard ratio (UHR) for overall mortality and for developing metastasis relative to 1975–1982 decreased steadily to respectively 0.61 and 0.25 in 1999–2006. It was noted however that the time to develop metastasis shortened, with the proportion of women developing metastasis within 5 years increasing from 66.3 to 83.9%. Furthermore, median survival following the development of metastasis decreased from 1.52 years in 1975–1982 to 0.94 years in 1999–2006. Filtering based on the St. Gallen criteria for high-risk patients and grade 3 tumours showed a subset of patients who developed metastasis early and died within a short time frame. These patients were identified across all four decades.
Discussion and conclusion: Improvement in survival and decrease in risk of metastasis over the past 40 years show that advances in treatment of primary tumours have made a significant impact. However, some women develop metastasis early and die early. This subset of women with poor prognosis, present in all four time periods, appears to be resistant to systemic therapy. Ongoing research is currently being conducted on the identification of a genomic-resistant signature in this subset of patients.

P5.7

Prevalence of germline mutations and the accuracy of self-reported family history of cancer in patients with early-onset breast cancer

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The main objectives in this cohort of early-onset breast cancer were to determine the frequency of germline mutations, as well as to evaluate the concordance between self-reported and registry-reported information regarding family history of breast cancer (BC), ovarian cancer (OvC), and other types of cancers in first-degree relatives.
Materials and methods: In the South Swedish health care region between January 1st 1970 and December 31st 2013, 779 women were diagnosed with BC before age 36. Out of these 779, 231 were registered for genetic counseling at the Oncogenetic clinic at Skåne University Hospital, Lund, Sweden. Information regarding germline mutation status as well as information of self-reported and registry-reported first-degree family history of cancer was collected.
Results: Of the 231 early-onset BC patients registered at the Oncogenetic Clinic, 224 were mutation screened which revealed germline mutations in 30.4%. The mutations were dispersed as follows: 18.8% *BRCA1*, 7.1% *BRCA2*, and 4.5% germline mutations in other genes, respectively. Almost perfect agreement was observed between self-reported and registry-reported information regarding first-degree family history of BC ($\kappa = 0.92$) and OvC ($\kappa = 0.86$). Lesser agreement was observed regarding family history of other types of cancer ($\kappa = 0.51$).

Conclusions: The prevalence of mutation carriers was higher than expected. It should be noted that the true prevalence of mutation carriers of cancer-predisposing genes other than *BRCA1* and *BRCA2* in our study is unknown, and that the prevalence of mutations could be even higher than reported. Our results demonstrate that physicians and genetic counselors can rely on self-reported information regarding BC and OvC in first-degree relatives. However, self-reported information regarding other types of cancer is not communicated as effectively. Furthermore, we observed that even though all BC patients fulfilled the criteria for genetic counseling and testing by being younger than 36 at diagnosis, a large number of these early-onset BC patients were not registered at the Oncogenetic clinic and thereby did not receive genetic counseling at the Oncogenetic Clinic. This finding merits further elucidation.

P5.8

Breast cancer risk information engages women with a breast cancer prevention weight loss programme

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Background: The PROCAS Lifestyle Study is assessing the best ways to engage women with behaviour change interventions to reduce their risk of breast cancer and other diseases. It is testing whether uptake and retention are influenced when women are told of their breast cancer risk, and how effective a breast cancer prevention programme (BCPP) is compared to a multiple disease prevention programme (MDPP) that additionally includes personalised risk information on CVD and diabetes.
Methods: Overweight (BMI $\geq$ 25 kg/m^2) NHS Breast Screening Programme attendees identified as high, above average, average or low breast cancer risk were invited to two different phone and web programmes; either the BCPP or the MDPP. Phase 1 recruitment invited women once they had been informed of their individual breast cancer risk (BCPP $n = 45$, MDPP $n = 81$). Phase 2 invited women who had not yet received their risk information (BCPP $n = 26$, MDPP $n = 26$), who subsequently received risk feedback during the programme.
Results: Phase 1 reported significantly higher uptake in high/moderate risk women (23%) compared to low risk (5.2%; $p < 0.0001$), but did not influence drop-out (high/moderate risk 15% versus low risk 16%). Phase 2 had a higher drop-out amongst low-risk women after they received information about their low risk (61.5% vs high/moderate risk 11%; $p = 0.0004$). The BCPP and MDPP weight loss programmes were equally effective across all risk groups. Baseline observation carried forward analyses at 12 months found 58% of the MDPP and 57% of BCPP groups maintained a weight loss of $\geq$ 5%.
Conclusion: Breast cancer risk information influences uptake to and engagement with a breast cancer prevention weight loss programme. Additional CVD and diabetes health risk information were acceptable, but did not increase uptake or weight loss success with a BC prevention programme.

P5.9

Randomised controlled trial of continuous versus intermittent energy restriction during adjuvant chemotherapy (The B-AHEAD 2 Trial)

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Background: Observational data indicate excess weight at breast cancer (BC) diagnosis and weight gain during adjuvant chemotherapy increases risk of recurrence and death[1]. We have demonstrated that continuous energy restriction (CER) and exercise are only partially effective at limiting the usual 2.5–3 kg weight gain which occurs during chemotherapy. Our randomised trials in healthy subjects indicate that intermittent energy restriction (IER) is equivalent or superior to CER for weight control. Here, we will report the results of a randomised comparison of IER versus CER amongst 172 women receiving adjuvant chemotherapy.
Methods: Participants followed an IER or CER throughout the 4.5- to 6-month course of adjuvant/neoadjuvant chemotherapy. The primary end points were body weight, body fat and lean body mass assessed with DXA. Secondary endpoints were chemotherapy toxicity (self-reported CTCAE, Cytokeratin 18 and FMS Like Tyrosine Kinase 3 ligand serum markers), quality of life (FACT scales) and serum markers associated with prognosis (insulin sensitivity, adiponectin, leptin).
Results: Women were recruited immediately after surgery (39% uptake); 86 were randomised to IER and 86 to CER. There was an 84% retention to the trial. Reduction in weight was greater with IER versus CER; mean weight change (95% CI) $-$ 2.07 ($-$ 2.93, $-$ 1.21) versus $-$ 0.70 ($-$ 1.48, 0.07) kg, $p = 0.02$, and reductions in body fat tended to be greater with IER; $-$ 2.1 ($-$ 2.9, $-$ 1.3) versus $-$ 1.0 ($-$ 1.8, $-$ 0.29) kg, $p = 0.056$. Fewer of the IER group experienced severe toxicities when receiving adjuvant taxane therapy than the CER group; percentage of the groups with grade 3 or 4 toxicity were IER 18% versus CER 32% ($p = 0.036$).
Conclusions: High uptake and adherence reflects interest and motivation of women to make positive changes to lifestyle even at the time of diagnosis. The study suggests that IER is superior to standard CER for controlling weight and may also reduce severe chemotherapy toxicity.
(1) Chan et al. Ann Oncol. 2014 Oct;25(10):1901–1914.

P5.10

Clinical event reporting by active versus passive follow-up methods in the IBIS-II trials

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National registries are available to passively follow up 'flagged' clinical trial participants, allowing researchers to capture clinical events of interest for long-term follow-up. We compared events captured by 'active' ongoing trial follow-up methods in the IBIS-II trials (e.g. annual clinic visits at research site during treatment period and annual participant questionnaires in the follow-up period) with passive event rates obtained from data registries (e.g. cancer registry, Hospital Episode Statistics).

Datasets were requested for 2859 IBIS-II participants from NHS Digital and the NHS Wales Informatics Service for the time period 2003–2016. Predefined clinical events of interest codes (related to primary and secondary objectives) occurring after randomisation were used as a search criteria and compared to known events in the existing IBIS-II database through automatic matching and also secondary review by a data manager. All new passive events were followed up for verification with the clinical research site or GP and additional data (receptor status, grade, size etc.) requested.

Clinical events identified through passive dataset review and agreement with existing events identified via active methods

Clinical event	Total passive events	Passive events already identified by active follow-up	New passive events
Cancer Registry (Breast Cancer and DCIS)	122	115 (94%)	7 (6%)
Cancer Registry (Other primary cancers of interest)	112	90 (80%)	22 (20%)
Mortality	90	77 (86%)	13 (14%)
Fractures	127	107 (84%)	20 (16%)
Cardiovascular/ thromboembolic events of interest	96	79 (82%)	17 (18%)
Total	547	468 (86%)	79 (14%)

Our data show active follow-up methods captured 94% of breast cancer and DCIS events (primary trial objective) reported in national registries, suggesting little additional benefit of completing passive and active follow-up concurrently. However, national registry review may add significant value in identifying secondary trial outcome measures (including other primary cancers of interest and deaths), where up to 20% of the total passive events had not been previously recorded.

P5.11

Introduction of Biennial Screening Regimen for women assessed in Breast Test Wales found to have benign proliferative breast changes (B3)

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Introduction: Vacuum-assisted biopsy (VAB) and excision (VAE) techniques have recently been adopted in breast screening in Breast Test Wales (BTW) and are routinely used as second line biopsy technique as an alternative to surgical open biopsy. Women with proliferative breast changes (B3) diagnosed on core biopsy, where malignancy was excluded with surgery, would previously have been returned to routine recall within the BTW screening programme or followed within the symptomatic service. The utilisation of VAB/VAE should reduce the number of surgical biopsies and have similar rates of upgrade to malignant diagnosis but there is no nationally accepted agreement on follow-up of B3 cases diagnosed on VAB/VAE. We reviewed previous cases as a benchmark against which to measure the new service.

Methods: To ascertain the rate of B3 biopsies within the BTW screening programme, the upgrade rate to malignancy and the risk of future breast cancer diagnosis, we retrieved screening episodes recorded in the BTW database between 2000 and 2015, where a core needle biopsy result had been documented as B3. The identified cases were then cross referenced with the Welsh cancer registry to ascertain the time interval between screening invitation and the date of diagnosis of breast malignancy. Cancers diagnosed within 6 months of screening were counted as upgraded from B3 to malignant and those more than 6 months considered as an interval or subsequent screen-detected cancer.

Results: Between January 2000 and December 2015, 1781 biopsies in the screening programme resulted in B3 pathology. 430 biopsies (24%) were upgraded to invasive/in situ disease on second/surgical biopsy. 1352 were returned to routine recall of which 276 (20%) have developed a breast malignancy since, ranging from 125 to 4574 days (12 years) since the "B3 screening appointment".

Discussion: VAB/VAE is a new technique and with the recognised increased risk of subsequent breast cancer, we propose a biennial screening regimen for women with proliferative breast change diagnosed through screening.

PATIENT CENTRED CARE

P6.1

Patients who refuse treatment of breast cancer—an ethical dilemma for the MDT

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Breast cancer is the most common female cancer. In the general population, there is heightened awareness through the media spotlight and celebrities' talking about their experiences. The national screening service has targeted women in a specific age group and has succeeded in bringing to the fore patients with impalpable breast cancer. However, the majority of patients are those who find lumps or other symptoms and present to their GP. They are fast tracked under the 2-week rule and attend the one-stop clinics. They have good understanding of what to expect in the clinics through information sent by the hospital prior to their visit. The majority are agreeable to investigations involving mammogram/ultrasound and needle biopsy. All NHS units have breast care nurses to offer support to the patients and their partners at the time of diagnosis and during discussion of treatment. The newly diagnosed cancer patients require treatment to commence within an allocated time frame to adhere to national targets.

Conventional breast cancer treatment options include surgery, radiotherapy, endocrine, treatment and chemotherapy. The majority of women wish to have the treatment commenced urgently and this coincides with the hospitals requirement to meet the waiting time and start of treatment targets.

Patients and method: We submit case reports of six patients who refused treatment and their outcome. All patients attended their appointments up to the point of diagnosis. Subsequently, three refused treatment at the outset, two sought to delay treatment and one was accepting treatment in part. Four patients were kept under review. Two patients changed their mind during the course of the visits and went on to have surgery.

Discussion: These patients formed an ethical dilemma for the treating MDT. Our team discussion revolved around groups who felt that discharging patients who refuse treatment was inevitable, whilst those opposing this offered vigorous counter arguments that these patients should be deemed as most vulnerable and needed to re-attend the clinic more frequently to allow further discussions. It was proposed that these patients also needed to see different members of the MDT team to allow input form varying specialities. All our patients were seen by more than one specialist including oncologist, by breast nurses, radiologists and were given the option to talk to other patients and counsellors. Keeping these patients under review gave the unit the opportunity to understand the reasons behind their refusal and the ability to address their concerns and enhance the understanding of their condition.

The 'success' with two patients changing their mind and accepting treatment indicates the need for further debate and for national guidelines for breast teams to manage these patients.

P6.2

Taxane Chemotherapy and Nail Toxicity in women with breast cancer: an evaluation of interventions

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Nail problems arising from chemotherapy-induced toxicity can vary in appearance, severity, and may have a significant impact on quality of life (QoL). Despite a higher number of nail changes in taxane regimens, nail problems are often under-reported in studies involving taxanes and fail to provide adequate descriptors of nail problems experienced. There is currently no evidence regarding effective interventions to manage or treat nail problems other than the use of frozen gloves. Standard care is lifestyle advice plus/minus nail oil. Anecdotal evidence suggests that wearing dark nail polish throughout treatment may provide a beneficial effect. However, no evidence exists to support this hypothesis. This study aimed to provide initial evidence by exploring this widely held assumption in female breast cancer patients about to receive taxane chemotherapy. This randomised trial aimed to assess the extent of nail problems at three time points: initial chemotherapy cycle (baseline), 3 weeks (follow visit 1) and 3 months (follow visit 2) post completion of chemotherapy. One hundred and five women were recruited and randomised to receive either standard care, or standard care with dark nail polish **or** specialist nail drops®.

Clinicians assessed nails using an innovative tool, the NToX-G12. Medical images were captured at each study visit. To investigate the inter-relationship between nail grade severity and QoL, participants also completed the NToX-QoL tool at each visit to assess the impact and severity of any physical, functional or emotional change as a direct result of any nail problems. End of study feedback evaluated acceptability and satisfaction of interventions. Interim analysis demonstrates changes in QoL and severity scores to suggest the clinical advantage of the interventions. Almost 70% were receiving adjuvant treatment with 33% on Herceptin. Twenty-six participants withdrew as a result of disease progression. Full statistical results are pending and will appear in our final presentation.

P6.3

Patients and surgeons can safely choose implant- ADM/mesh reconstruction following previous radiotherapy to the breast; a retrospective cohort study

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Introduction: The need for reconstruction in a breast that has previously undergone radiotherapy is increasing, with woman often keen to explore immediate implant- ADM/mesh-based reconstruction. Our retrospective cohort aims to show it if feasible and safe in selected women.

Methods: Single-unit reconstructive database was used to identify all women who had undergone implant-ADM/mesh reconstructions having previous radiotherapy to the ipsilateral breast. Full case-note review was performed

Results: Twenty-one women met inclusion criteria, undergoing skin-sparing mastectomy and immediate reconstruction between 2010 and 2017; 19/21 (90%) achieving a successful reconstruction with implant in situ to date, mean follow-up of 28 months. Mean age at reconstruction was 59 years with 20/21 women deemed ASA1-2. The majority of women were non-smokers (17/21) with mean interval of 11 years (range 2–34 years) from radiotherapy to reconstruction. Recurrent DCIS or invasive disease was the most common reason for mastectomy (13/21); other reasons included risk-reduction and cosmesis. Fourteen women had a variable-volume device placed with mean volume of 270 cc at insertion (range 0–460 cc); pocket-drains

and antibiotic cover were routine. Most common adjunct used was STRATTICE (10/21); dermal-sling, BioDesign & TiLoop were also used. Post-operative seroma requiring drainage occurred in 6/21 women and 6/21 had delayed wound-healing beyond 2 weeks. One patient lost the implant due to infection and 1/21 for poor cosmesis. Five women had skin-flap redness during the healing period. No apparent themes were identified in the 2/21 woman who lost implants; both underwent successful salvage with autologous tissue. Ten women underwent unplanned further procedures, most commonly lipomodelling (8/21).

Conclusions: Implant-based reconstructions are a safe option after previous breast radiotherapy; surgeons need to be highly selection on whom to offer this to and be observant to common post-operative events such as redness, seroma and slower wound-healing. Radiotherapy skin change may increase the need of further procedures such as lipomodelling.

P6.4

Patient Advocates are Partners in Global Breast Research: value is recognised by early advocate involvement in the PREvent ductal Carcinoma In Situ Invasive Overtreatment Now (PRECISION) study and related trials

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Background: Early introduction and international collaboration of patient advocates is essential in the investigation of safe treatment of DCIS. This diagnosis has increased since the introduction of screening. The treatment of low-risk DCIS has been the subject of debate, controversy, anxiety and cost to women and health services globally. There has been much "public" as well as "professional" discussion leading to considerable distress. Some surgeons have concerns about carrying out unnecessary surgery on patients with low-risk DCIS, but this is current best practice due to lack of credible evidence that monitoring can be safe. Some women feel their "lives were saved" whilst others feel "mutilated" by "unnecessary" surgery. These women cannot know the true harm or benefit of the treatment which they receive without evidence from clinical trials and biological/molecular research.

Aims

- To provide evidence of the value of patient involvement.
- To encourage recruitment in "difficult to recruit studies" by educating the public, patients and clinicians.
- To assist in the design of effective information tools.
- To increase liaison between advocates internationally to understand differences in culture, health services and patient expectation.

Methods: The PRECISION team includes two patient advocates from each of the DCIS randomized trials (LORIS in the UK, LORD in the Netherlands and COMET in the US).

Our group will liaise with other groups involved in the wider efforts around DCIS. We will take part in media interviews and public debates to raise awareness and the need for evidence to change practice to reduce overtreatment of low-risk DCIS. We will work with our researcher colleagues in each PRECISION work project, join the PRECISION steering group, and liaise together via regular calls. Importantly, we will encourage awareness amongst clinicians to emphasize that patients wish to be informed about available trials, and that not to do so is denying patient choice.

Results: An increase in interest and recruitment can be measured and the influence of early involvement of patient advocates can be demonstrated so that the model can be used in other trials. The biology is intricate, scientific and exciting but it is crucial that the outcome is available and understood by all women and their physicians worldwide. The results will be promoted by patient advocates through publications, social media and patient groups. Advocates will appear as co-authors on scientific publications.

Conclusions: With the increase in international clinical trials, there is a need for further understanding of the differences in practice and patient need in different hospitals—as well as in different countries. We will show the value of our collaboration by demonstrating the results of patient advocate involvement in the PRECISION program.

* The PRECISION Team is a Cancer Research UK Grand Challenge Award 2017 winning team and will be jointly funded by Cancer Research UK and the Dutch Cancer Society

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P6.5

Breast surgery under regional anaesthesia without the use of a general anaesthetic: implementation and patient satisfaction

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Thirty-two percent of women diagnosed with breast cancer are over 70 years of age. General anaesthesia (GA) becomes more challenging with advancing age due to frailty, comorbidities and patient choice. 74% of patients diagnosed with breast cancer undergo a surgical procedure, but only 25% in patients over 85 years of age.

We report the experience of patients who were listed for breast surgery under regional anaesthesia, from the implementation of this service in January 2016.

Twelve patients were identified, (median age 74, range 55–91), 11 were ASA III. Operations were four mastectomy ± axillary surgery, five wide local excision ± axillary surgery and three benign/diagnostic operations. In 11 (92%) patients, the surgery was started under a regional block. One underwent a GA, due to increased patient anxiety in the anaesthetic room. Two patients were converted to GA during the operation, because of patient distress. There were no recorded complications directly attributable to the regional anaesthesia. One patient recovered from her breast surgery, but collapsed and died on day 13 while awaiting a nursing home bed.

All patients who underwent surgery under a regional block were questioned. All (100%) stated that they would have a further procedure performed under regional anaesthesia. Seven (87.5%) patients stated they would recommend the technique to their friends and family.

Breast surgery under regional anaesthesia is a valuable technique, where a GA is unsuitable, with excellent patient satisfaction. In view of the relatively high conversion rate to GA, preoperative selection and optimisation need to be robust.

P6.6

Emotional speech during therapeutic radiography weekly review consultations in patients treated for breast cancer: implications for recurrence fears

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Fears of cancer recurrence are common and at high levels are detrimental to patient well-being and quality of life. Patients who experience substantial fears are more likely to suffer depression and have higher levels of health care utilization. Once these fears are established, they are difficult to change. This study focused on the conversations during the weekly reviews of patients with breast cancer and their therapeutic radiographer staff. Previous work in health provider and patient communication on emotional expression in clinical interviews has shown that the clinician who responds by providing opportunity for the patient to express their concerns confers a psychological benefit to the patient in the longer term. The aim was to investigate the nature of interactions during the final phase of the treatment process for breast cancer patients and test if the response of staff to emotional content was associated with patients' recurrence fears. Audio recordings of over 90 patients were collected during the first two review sessions during radiotherapy treatment. The tapes were securely stored and analysed by using a well-validated coding scheme (VR-CoDES-P) which identifies emotional cues and concerns including the number of times that staff provided space or closed down further patient expression. In addition, fears of cancer recurrence were assessed using a seven-item questionnaire (FCR7) at each of these appointments and at 2-month follow-up. Overall, staff responded to emotional content with open-ended interaction. However, more detailed linear modelling demonstrated that the number of occasions that staff closed down patient discussion of emotional cues and concerns in the second session had a negative impact on recurrence fears at 2-month follow-up ($p < 0.05$). This work tentatively shows that the routine interactions of staff during treatment may have lasting psychological effects on patients. Implications for patient-centred care will be discussed.

P6.7

An audit of local experience with the Moving Forward Course and cancer survivalship

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Introduction: Breast cancer treatment can result in long-term side effects which can seriously affect quality of life. In our institute, we have joined The Moving Forward Course in conjunction with Breast Cancer Care (BCC) in 2015. We have audited our results for the first 2 years.
Methods: The moving forward course is evaluated by BCC through a pre- and post-course questionnaire. The questionnaire enables quantitative as well as qualitative measure of enhancing knowledge and awareness. Each questionnaire has nine questions with score ranging from strangely disagree (0) to strongly agree (5) and covers different well-being aspects with a space for free text comments.
Results: We had six courses over the last 2 years with 72 delegates. There was an overall improvement in score for every aspect covered by the course; good understanding of side effects (4.5 vs. 3.3), self-help, living better with and beyond cancer (4.6 vs. 3.1), knowledge about information and support (4.7 vs. 3.2), accessing breast cancer care and local services (4.7 vs. 3.5), keeping breast aware after treatment (4.3 vs. 2.7), confidence to live with and beyond cancer (4.2 vs. 3.2), feeling less isolated (4.4 vs. 3.2) and self-esteem (4.2 vs. 2.9). There was a marginal improvement in ability to talk to family and friends (3.8 vs. 3.3).
Conclusion: There is a need for holistic courses that cover breast cancer survivorship issues. These courses should be considered complimentary to the breast cancer treatment and have to be available to patients following their treatment.

P6.8

An audit on 2-week referrals to a one-stop breast clinic in a district general hospital

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Introduction: Clinics where 'triple assessment' for breast problems can be performed within the same day are referred as 'one stop clinic' or 'rapid access breast clinic'. Association of Breast Surgery Quality Indicator states that all patients with breast symptoms referred to a specialist are seen within 2 weeks of referral (national requirement = 93%). Although this system allows patients with suspected cancer symptoms to be seen rapidly, the effectiveness of this 2 week referral has been continuously questioned and criticised.
The aims of this audit were to ascertain that patients referred on two-week wait pathway are seen within 2 weeks and review the referral type for a number of patients (i.e. urgent or non-urgent) to determine if feedback on appropriate referral can be provided.
Methods: This was a retrospective audit looking at the numbers of patients referred using the 2-week referral over 12 months. This audit reviewed the percentage of 2-week referrals seen promptly. It also reviewed the percentage cancer diagnosis in these 2-week referrals.
Results: Over a 12-month period, 2174 patients were seen in clinic (Table 1); symptomatic breast cancer diagnosis was 5.9%. Most patients (98.7%) were seen within the 2-week period but there were few breeches (1.25%). The target was still met. There were many patients with non-urgent symptoms that were referred on the 2-week wait pathway.
Conclusions: The one-stop breast clinic in our hospital is efficient and meets the standard set by ABS. We suggest that perhaps breast clinicians should be given a role in vetting the 2-week referrals into urgent and non-urgent.

Table 1 Two-week referral to 'one-stop' breast clinic

Month	2-week wait referrals	Diagnosed cancers	Percentage of cases diagnosed with cancer (%)	2-week wait breeches (%)	Target (93%) (%)
April–July 2016	715	44	6.07	1.96	98
Aug–Nov 2016	717	45	6.3	0.89	99
Dec–Mar 2016	742	40	5.55	0.89	99
Total	2174	129	5.9	1.24	98.6

P6.9

Comparing clinician and patient perspectives in the management of hot flushes in UK breast cancer patients. NCRI Breast CSG Symptom Management Subgroup

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Women who have been treated for breast cancer identify vasomotor symptoms, such as hot flushes and night sweats (HFNS), as a serious problem. An estimated 550,000 people live in the UK today with a diagnosis of breast cancer and up to 70% experience HFNS. Oestrogen replacement remains the most effective treatment for hot flushes. However, this is contraindicated in the majority of women with oestrogen-dependent breast cancer.

Fewer than 50% of women with ER+ breast cancer complete the recommended 5 years of endocrine treatment—tamoxifen and AIs.

This lack of adherence, possibly due to unacceptable side effects, leads to a 20% excess breast cancer mortality.

Patient members of the National Cancer Research Institute UK Breast Clinical Studies Group identified that there is very little research into the management of symptoms after breast cancer treatment. In response, we established a Working Party on Symptom Management. We all have a particular interest in the management of HFNS, and members include patients; clinical and academic partners representing oncology, psychology, gynaecology, complementary therapies; and the voluntary sector.

We gauged current clinical practice of the management of HFNS by surveying breast cancer patients, GPs and health care professionals and we will present this data. There was a considerable mismatch between the three groups: e.g. 40% of patients reported that no HCPs or GPs had asked them about HFNS. Despite over 90% of GPs & HCPs reporting that they prescribed drugs to alleviate HFNS, only 26% of the patients had been offered drugs and fewer than 2% said they helped. 31% of the patients said that the HFNS were severe enough for them to consider stopping endocrine therapy. If women are to be helped to adhere to their life-saving treatment, new approaches need to be developed to ameliorate HFNS

P6.10

Evaluation of patient-centred communication in breast clinic; how are we doing and what we need to improve?

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Background: is good quality evidence that effective communication can lead to enhanced patient care and increased compliance. Effective patient-centred communication is vital in rapid access breast clinics. Health care professionals (HCP) need to demonstrate focused active listening, express empathy, and understand the patient's perspective regarding the illness and various treatment choices. The communication skills that promote shared decision making empower patients to make right treatment choices for themselves. We evaluated the communication effectiveness of the HCP in the breast clinic using Medical Interview Satisfaction Survey-21 MISS 21.

Methods: Fifty-one consecutive patients were invited, in this pilot phase of the study, to complete MISS-21 after consultation in a new patient rapid and follow-up breast clinic. Healthcare Professionals also rated their satisfaction with the clinical consultation on the domains of rapport, distress relief, communication comfort, compliance intent and professional behaviour. All responses were recorded on Likert score 1–7.

Results: Our results suggested that patients had confidence in the clinical competence of the HCP (median 6, max 7). Most patients felt understood by their HCP (median 6, max7). The median score for explanation for the tests required and treatment need was 5.5. The overall score for distress relief and rapport were concordant (median 6) for both patients and HCP. Thirteen percent of patients felt that their consultations were rushed and 3% reported that they were given too much information.

Conclusions: Overall, patients are satisfied with the consultations in the new and follow-up breast clinics. However, the art of finding the right balance of giving adequate information which is right for the patient needs development. We plan to extend this evaluation to all HCP in our department. To address the communication skills developmental needs, neurolinguistics programming may be helpful.

Acknowledgement for the contribution of consultant and specialist nursing colleagues for their support and constructive feedback.

P6.11

Concordance between the HCPs' and patient's perception of the efficiency of the clinical consultation in breast clinic, scope for improvement

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Introduction: The psychological stress of patients attending breast clinic can be managed by effective communication. Healthcare professionals (HCPs) in breast clinics need outstandingly professional patient-centred communication skills for effective and communication to build rapport, relieve distress and promote compliance. The objective of the pilot project was to assess the suitability of an adapted version the' Medical Interview Satisfaction Scale' (MISS 21) to assess the concordance between the HCPs' and patient's perception of the efficiency of the clinical consultation.

Methods: In this pilot study, 50 patients, two clinicians, one specialist Breast cancer nurse took part. HCPs and patients assessed the clinical consultation on five subscales Distress Relief, Communication and comfort, Rapport, compliance intent and Behaviour on a 7-point Likert score. Two questionnaires had to be discarded as patients scored all questions as 7 or yes.

Results: There was a general concordance between the HCP and patients in satisfaction with the clinical consultation. The median score for HCP in the domains of communication comfort and rapport was 6. The median score for distress relief was marginally lower in the trainee doctors. HCP and patients found MISS-21 as an effective tool in assessing the consultations in Rapid access and follow-up

breast clinics. However, both patients and HCP found MISS-21 unhelpful to assess cancer diagnosis giving consultations.
Conclusion: MISS-21 is a useful tool to assess the quality of clinical consultations in Rapid access breast clinic and can help identify the levels and developmental needs of key skills as ability to develop rapport and provide distress relief. These skills can be taught and learned.

P6.12

Prospective Study of methods of Lymphoedema diagnosis and new Composite index for diagnosis

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Lymphoedema develops after surgery in 30% patients. A prospective, multi-centre NIHR study (UKCRN_ID_8881) compared multi-frequency bioimpedance spectroscopy (BIS, ImpediMed) with a validated perometer arm measurement to (1) determine which test has the best diagnostic accuracy, (2) identify the factors predicting development and (3) develop a composite index for lymphoedema diagnosis.

Participants ($n = 1100$) undergoing axillary clearance at nine centres had pre-operative and post-surgery (1, 6, 9 and 12 months, then 6) arm measurements by perometry, BIS (L-Dex), FACT-B+ 4 and lymphoedema checklist questionnaires. Relative arm volume increase (RAVI) of $> 10\%$ diagnosed lymphoedema with sleeve application a secondary endpoint. Predictors of lymphoedema were determined using logistic regression, optimal diagnostic method using diagnostic accuracy, and changes in QoL assessed using GEE.

Median age was 56 (range 22–90) years; 78% received radiotherapy and 65% chemotherapy. By 24 months, lymphoedema was detected in 21.4% of women by perometry, 39.4% by BIS and 24.4% by sleeve application. Diagnostic accuracy for RAVI $> 10\%$ and BIS compared to sleeve application was 94 and 88.9% respectively. BIS compared to RAVI $> 10\%$ had a 91% (95%CI 88–93%) diagnostic accuracy at 24 months.

A predictive model for risk of lymphoedema (AUROC 0.80) including RAVI measurement ($p < 0.001$), number of positive nodes ($p < 0.001$) and BMI ($p = 0.015$), all allotted a points score was developed. A composite definition of lymphoedema comprising a RAVI > 9 and self-reported "considerable" swelling had a diagnostic accuracy of 94% or greater at 6, 12 and 24 months.

Initial decreases in QoL scores post-surgery were larger and took longer to return to baseline values in lymphoedema patients. Poorer FACT-B+ 4 QoL scores were associated with smoking ($p = 0.018$), high BMI ($p < 0.001$), chemotherapy (at 6 months $p < 0.001$) and age ($p < 0.001$).

Increases in arm volume $> 5<10\%$ by 6 months predicts a 35% risk of lymphoedema by 24 months. Lymphoedema led to lasting quality of life deficits.

P6.13

Feasibility and acceptability of *Accepting your Body after Breast Cancer:* A body image intervention for women who have undergone treatment for breast cancer

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Objective: A major consequence of treatment for breast cancer is changes to appearance. These can include breast asymmetry, scarring, lymphedema, hair loss and thinning, weight fluctuation, skin and nail discolouration, and dermatitis. These alterations can impose a negative and enduring impact upon the body image of breast cancer survivors. However, body image interventions for this group are lacking in empirical support. The aim of the study was therefore to assess the acceptability, feasibility and preliminary effects of 'Accepting your Body after Breast Cancer', an evidence-based intervention for women in midlife adapted to promote positive body image among breast cancer survivors.
Methods: The study adopted a single arm pre-test, post-test, and 1-month follow-up design. Twenty-three breast cancer survivors were enrolled (M age $= 51.55$ years; M time post-treatment $= 2.62$ years) and formed two groups. All participants received the 7-week Cognitive Behavioural Therapy intervention, co-facilitated by a clinical psychologist and peer. Observational and self-report data on intervention acceptability and feasibility were collected. Intervention efficacy was evaluated by assessing changes in the primary outcome of body image (e.g., body appreciation, weight and shape concern) and various secondary outcomes (e.g., distress, self-esteem).
Results: Participant evaluations of the intervention suggested that it was both acceptable (e.g. 94% found it beneficial and would recommend it) and feasible (e.g. 87% completed the intervention; 80% of these completed post-intervention and follow-up assessments). Improvements were identified at either post-test or follow-up in nearly all body image measures. Maintained improvements were attained in body appreciation, weight and shape concern, acceptance of ageing-related appearance changes, and self-esteem. Further, small-to-large effect sizes were identified in the majority of primary and secondary outcome measures.
Conclusions: This study suggests that the adapted intervention is acceptable, feasible, and demonstrates preliminary efficacy in improving body image and secondary outcomes among breast cancer survivors. The next step of evaluation is to conduct a randomised controlled trial to explore the longer-term impact of the intervention.

P6.14

Surgical outcome measures following mastectomy and breast reconstruction in a cohort of patients at high risk of breast cancer

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Introduction: Many women with breast cancer-specific gene mutations (e.g. BRCA) or otherwise at high risk choose to have risk reducing mastectomy (RRM) with/without breast reconstruction (BR). No publications have reported surgical outcomes specifically for this patient group. We present surgical outcome measures (SOMs) in patients undergoing RRM with/without BR.

Method: Casenotes of women undergoing RRM from the Manchester Family History clinic (1987–2014) were interrogated for SOM which were compared between those with and without a cancer diagnosis. Some women were diagnosed with breast cancer immediately prior to RRM and some diagnosed incidentally at RRM (preoperative screening negative).

Results: Cancer was diagnosed in 92 women (21%; cancer group, CG), 353 (79%) had no cancer diagnosis (benign group, BG). Women were older in the CG than BG (mean 43.3 and 39.0 years, respectively). Length of follow-up from first reconstruction was similar [median 73.0 (CG) v 70.0 (BG) months]. More BG than CG women had nipple-preserving mastectomies ($p < 0.001$). Immediate implant-based reconstruction was commonest in both groups. Mean number of planned surgical procedures was higher in CG (2.85) than BG (2.41), $p = 0.01$. Both groups had similar numbers of unplanned and emergency procedures.

Incidence of complications did not differ significantly between groups (CG 19.4%; BG 22.6%). The commonest emergency procedures were implant removal (CG 5.6%) and haematoma evacuation (BG 4.7%). Median cumulative inpatient length of stay did not differ but CG required a higher number of outpatient clinic visits (CG 18; BG 15; $p = 0.03$). Longevity of reconstruction (i.e. time to first revision) did not differ between groups [median 43 (CG) v 55 (BG) months]. One of the BG subsequently developed breast cancer 197 months following RRM.

Discussion: Our unique data show that RRM is safe and effective but multiple operations and outpatient visits may be required, especially in those found to have malignancy. This needs to be discussed at screening.

P6.15

Patient information materials; the use of co-design and collaboration

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As a consultant nurse for breast cancer, I have always played an active role in patient support. With the anticipated introduction of a new breast cancer drug for hormone-receptor-positive, HER2-negative metastatic disease in the UK, a group of consultant cancer nurses worked in partnership with a pharmaceutical company, a patient information support manager and a patient writing/reading panel in the development of the patient materials.

As a group, we supported stage-one development of the materials to ensure that the scientific language, tone and format was appropriate for patients. Early input from patients to associated materials is vital to ensure that they are truly user-oriented and provide the highest quality information to our patients, with language that informs, supports and comforts them.

There is no better way to test patient materials than discussing them with patients. Unfortunately, many companies miss out this vital review. We conducted an evening focus group, stage two, to provide input and validation to these materials. This stage was an informative and essential part of the development process. Subsequently, the company had these materials reviewed by the Medicines Health Regulatory Agency (MHRA) receiving zero amendments, demonstrating the value of this two-stage process and co-design in producing high-quality materials. We hope to see that joint collaborations lead to an improved patient experience on treatment and improved side-effect management and adherence during a difficult and overwhelming time for our metastatic breast cancer patients.

P6.16

Use of a combination of modules of BREAST-Q in lateral chest wall flap partial breast reconstruction: a tale of two cities

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Introduction: Lateral chest wall perforator flap (CWPF) provides a non-muscle parenchymal replacement option for partial breast reconstruction following breast conservation surgery (BCS) in high tumour:breast ratio excisions. Since this is the latest oncoplastic procedure in the armamentarium of breast-conserving surgery options, there is not yet a specific Breast-QÓ module. Hence, a previous single centre pilot study explored a combination of two modules of Breast-Q (Abstract, ABS, 2016). This study combines pilot data from two University teaching hospitals.

Methods: The Breast-Q provides a Q-score that ranges between 0 and 100, with 100 being the highest score in both patient satisfaction and Quality of Life (QOL) domains. Patients from two University teaching hospitals completed a combination of post-operative breast conservation therapy (BCT) module and post-operative LD flap module of Breast-Q.

Results: Mean post-operative Q-scores derived from 36 available responses were as follows:

- Satisfaction with: breast = 81.3, irradiated breast = 87.2, patient care, surgeon and staff > 95
- QoL domain: physical well-being = 76.4, psychosocial well-being = 82.1, sexual well-being = 60.4
- Back appearance = 90.5, shoulder and back function = 77.1

Conclusions: Where displacement option such as therapeutic mammoplasty (i.e. BCT with reduction) is not applicable, CWPF offers an alternative BCS option (often avoiding mastectomy) especially in smaller breasts with no or low-grade ptosis. Our two-centre cohort exploring patient-reported outcomes finds favourable patient satisfaction scores in this latest Oncoplastic procedure. Further, this study reinforces the feasibility of combination of two Q-score modules though needs validation in a larger multi-centre prospective study.

P6.17

The NT0X-G12- A new clinical assessment tool for nail problems as a result of taxane chemotherapy regimens

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Nail problems associated with chemotherapy are under reported in terms of the incidence and severity despite an increasing number of nail changes in those receiving taxane regimens. Furthermore, little or no descriptors of the actual type of nail changes are ever provided.

Nail toxicity can vary in appearance, severity and impair function. Reported problems can range from pigmentation to onycholysis. Present practice to measure chemotherapy toxicities, including nails, utilises the Common Toxicity Criteria for Adverse Events (CTCAE). However, this tool has previously been criticised for discrepancies when compared with information collected from patients and for being insufficient to accurately report the range and severity of potential adverse effects.

Currently, there is no detailed clinical assessment tool to capture the range of nail changes that patients with breast cancer may experience due to taxane chemotherapy. Our multidisciplinary research breast group developed a clinical tool, the NToX-G12 to assess nail toxicity in patients receiving taxane chemotherapy for breast cancer. This has previously been tested for content and face validity, and intra-rater and inter-rater reliability. The NToX-G12 scale incorporates visual images and is accompanied by a clinical master guide with matching visual images.

This innovative tool has been further tested in a randomised trial involving 105 females with breast cancer about to commence taxane-based chemotherapy. In addition to routine assessment using the CTCAE v3.0, clinicians used this new measure to assess and grade the nail condition of women at 3 time points: beginning of chemotherapy treatment, 3 weeks and 3 months post-completion of chemotherapy cycles. Medical images were captured at each visit. With final results pending, we will be able to report specific findings in our presentation.

P6.18

Evaluating taxane chemotherapy-induced nail problems on quality of life in female patients with a diagnosis of breast cancer

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Introduction: Nail changes associated with Taxane-based chemotherapy regimens have generally been under reported in terms of incidence and severity partially due to inadequate documentation, selective reporting and absence of non-specific nail outcome measures. Associated nail toxicities not only vary in appearance, severity and function, but may also limit activities of daily living (ADLs). For female patients, cosmetic and functional interference with normal ADLs may therefore greatly impact on quality of life and well-being. Currently, there is no specific outcome measure to capture the patient perspective on the impact of nail changes caused by taxane chemotherapy regimens. Existing measures are limited to specific dermatological toxicities such as psoriasis. Our team has developed a generic, 18-item quality of life tool for patients, the NToX-QoL questionnaire. This has been previously tested for content and face validity, inter- and intra-rater reliability (Stage One).

Aims: To test the face validity, and inter-rater reliability of the NToX-QoL in a large sample. To investigate the correlation between the NToX-QoL and the generic EQ-5D-5L measures.

Method: An innovative tool, the NToX-QoL, previously tested for validity and reliability has been further tested in a randomised trial involving 105 women with breast cancer. The trial tested two interventions thought to ameliorate the incidence and severity of taxane chemotherapy-induced nail problems against standard care, and any associated impact on quality of life. The NToX-QoL and EQ-5D-5L questionnaires were completed by participants at three time points: beginning of taxane chemotherapy, 3 weeks, and 3 months post completion of chemotherapy.

Results: The NTox-Qol measure captured rich data that allowed physical and functional subscales to be tested against assessments of nail problems using the NT0X-G12 tool, and compared with scores from the generic EQ-5D-5L. Interim analysis provided further validation the QoL was sensitive to capturing the impact of treatment-induced nail problems. Full results are pending so will appear in the final presentation.

P6.19

Does pre-surgical anxiety impact on patient-reported outcomes following mastectomy and breast reconstruction in patients at high risk of breast cancer?

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Introduction: Women with genetic mutations specific to breast cancer (e.g. BRCA) or otherwise at high lifetime risk (> 30%) of breast cancer are routinely offered risk-reducing mastectomy (RRM) with/without breast reconstruction (BR). During their surgical decision-making process, the Hospital Anxiety and Depression Scale (HADS) is administered during psychological consultation. Some women may experience increased anxiety when facing the threat of breast cancer.

The Breast-Q is a patient-reported outcomes (PROMs) questionnaire specifically for patients who have had breast surgery, examining its impact on their physical, psychological and social well-being. There is no published research which has examined PROMs in relation to pre-surgical anxiety.

Method: The Breast-Q was administered to women who completed the HADS and had RRM and BR. Responses were compared between those with confirmed BRCA gene mutations, those confirmed not to have BRCA mutations, and a group of untested women.

Results: 128 women completed HADS; 81 (63%) had confirmed BRCA mutations, 9 (7%) were confirmed to have no BRCA mutation and 38 (30%) were untested. 41% of the BRCA group and 45% of the untested group revealed borderline or clinical levels of pre-surgical anxiety.

PROMs were generally similar across groups, with equivalent satisfaction with overall outcome, at 75%. The BRCA and untested groups tended to show the greatest contrasts in scores relating to physical well-being, e.g. overall satisfaction with breasts (61% vs. 56%), satisfaction with nipples (50% vs. 45%), and in satisfaction with information (77% vs. 70%). All groups reported 100% satisfaction with their surgeon, medical staff and office staff.

Discussion: In our study, women at high lifetime risk of breast cancer but with unconfirmed genetic mutation status suffered greater pre-surgical anxiety than those whose genetic status was confirmed. Post-surgical satisfaction was consequently affected, revealed by lower levels of physical satisfaction and satisfaction with information. This finding suggests the importance of addressing anxiety pre-surgery, particularly in the unconfirmed genetic mutation group.

P6.20

Patient-reported outcomes following mastectomy and breast reconstruction in a cohort of patients at high risk of breast cancer

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Introduction: Breast-Q is a patient-reported outcomes measure (PROMS) questionnaire specifically for patients who have had breast aesthetic/reconstruction surgery, examining the impact of breast surgery on women's physical, psychological and social well-being. The Manchester Breast Family History service is one of the largest in Europe. We uniquely examined Breast-Q PROMS in a large cohort of women who have undergone risk-reducing mastectomy (RRM) and reconstruction because of a high lifetime risk of developing breast cancer.
Method: Patients were identified from the Manchester Family History clinic database who either had therapeutic (cancer group, CG), or risk-reducing (benign group, BG) mastectomy and breast reconstruction between 1987 and 2014. Women in the CG group were diagnosed with cancer immediately prior to, or at the time of, RRM (i.e. preoperative screening tests negative). Breast-Q PROMS were issued to these patients and descriptive statistics of the two groups compared using a two-sided Mann–Whitney test.
Results: The CG had lower satisfaction with their breast reconstruction ($p = 0.04$) but equal satisfaction with the overall process of reconstruction surgery, compared to BG group. Wider psychosocial well-being was equivalent between the two groups but more intimate sexual well-being was significantly lower in the CG group ($p = 0.01$). More women in BG had nipple-preserving surgery ($p < 0.001$), reflecting a greater satisfaction with their nipples postoperatively ($p = 0.02$). Satisfaction with the surgeon, with medical staff and office staff was equivalent between the two groups.
Discussion: We note a decrease in satisfaction with the aesthetic appearance of breast reconstructions in the cohort of women diagnosed with breast cancer prior to or at the time of RRM. This is reflected in reduced well-being in intimate settings but not in a wider psychosocial setting.
These considerations should play a role in the counselling of women considering RRM.

P6.21

Does size matter in selection for oncoplastic surgical options in comparison with standard wide local excision?

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Aims: The pre-operative tumour radial dimensions often determine the choice between standard wide local excision (WLE) and oncoplastic breast surgery (OBS). We reviewed the 3-dimensional interplay between the two cohorts.
Methods: Single oncoplastic surgeon data for 70 randomly selected WLE or OBS procedures were statistically compared using the Median test (k samples) and Chi-squared test.
Results: The median age did not differ significantly between the two cohorts, 65 (54–70) for WLE versus 60 (48–66) for OBS, $p = 0.632$.

	WLE ($n = 35$)	OBS ($n = 35$)	p value
Boost radiotherapy (%)	17.1	31.4	0.163
Pre-operative tumour size (mm)	12.0 (10–17)	22.5 (15.8–28.3)	0.001*
Post-operative tumour dimensions (mm)			
Medial–Lateral	12.0 (8.0–20.0)	20.5 (12.0–26.8)	0.010*
Superior–Inferior	13.0 (8.0–20.0)	21.5 (14.0–25.5)	0.002*
Anterior–Posterior	11.0 (7.0–18.0)	15.0 (10.0–20.0)	0.185
Post-operative specimen dimensions (mm)			
Medial–Lateral	50.0 (40.0–65.0)	118.0 (82.0–179.0)	<0.001*
Superior–Inferior	44.0 (35.0–60.0)	109.0 (84.0–130.0)	<0.001*
Anterior–Posterior	25.0 (20.0–37.0)	56.0 (41.0–76.0)	<0.001*
Specimen weight (g)	31.0 (17.6–44.6)	72.1 (41.9–184.1)	<0.001*

Conclusions: Despite no age or radiotherapy bias, the median pre-operative 'tumour' size was significantly larger in OBS (by $\sim$ 70%) and concordant with post-operative 'tumour' radial dimensions. In contrast, median 'tumour' antero-posterior dimension was not significantly larger but 'specimen' antero-posterior dimension was double in OBS. This reaffirms objectively that although the need for OBS arises due to tumour radial dimensions, a significant proportion of the excision volume, in at least displacement type of OBS, is simply due to the pertinent cylindrical excision in the third dimension.

P6.22

Early breast cancer follow-up—the patients' perspective

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Introduction: Mammo-50 has the opportunity to gather patients' perspective on follow-up via the qualitative sub-study (QSS) and by working with Independent Cancer Patients' Voice (ICPV).

Methods: **Mammo-50 QSS:** Focus groups were carried out at Wythenshawe, Torbay, Birmingham and Shrewsbury to gather patients' experiences and perceptions of follow-up, and inform the topic guide for further in-depth, semi-structured telephone interviews from Mammo-50 participants. Ethics and consent were obtained for audio-recordings.

ICPV follow-up survey: ICPV members designed a survey to collect the follow-up experiences of patients with a diagnosis of cancer. The survey looked at patient experiences, unmet needs and preferences for follow-up after treatment. The online Survey Monkey tool was used to pilot the survey and, after modifications, ethics obtained to distribute this through the NCRN.

Results: To date, 6 focus groups have been carried out and over 20 individual interviews with Mammo-50 patients; patients in general were satisfied with their care and happy to be in a trial.

To date, 118 people who took part in the ICPV online survey stated that they had a diagnosis of early breast cancer. Over 2/3rds of respondents said they had some unmet needs during their follow up period; these were varied and included both physical and psychological needs. Additionally, some respondents declared that they would have preferred a different form of follow-up to that which they were prescribed/offered.

Conclusions: Mammo-50 QSS patients were in general happy with their care. However, patients asked for their opinions through the ICPV questionnaire indicated that follow-up after treatment for early breast cancer is not always as successful as it might seem. When given the opportunity to report unmet needs of any type, patients often report things which could be causing them distress but which may go unnoticed in routine follow-up.

P6.23

The big breast cancer conversation—a patient focused insight project to understand patient experience, needs and motivations in breast cancer

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The *Big Breast Cancer Conversation* is a UK wide study of over 2000 people set up with the purpose of gaining an understanding of how stage in breast cancer journey shapes experiences, needs, and motivations of patients. The need for the research was for the charity Breast Cancer Now to use this to shape how they communicate with and involve people affected by breast cancer most effectively. It was also to identify where collaboration with health care providers, researchers and the wider breast cancer community is required to approach breast cancer experience more holistically.

The project phases were as follows:

1. **In-depth face to face research** (20 women):

- Women currently receiving treatment for primary breast cancer
- Women no longer in active treatment for primary breast cancer
- Women with a secondary breast cancer diagnosis

2. **Reconvened in-depth interviews and focus groups** (40 people):

- Partners of women with a breast cancer diagnosis
- People with close family or friends affected by breast cancer
- Women 'at risk' of breast cancer due to gender and age

3. **Online survey completed by 2000 women and men UK wide** with a range of experiences.

The research provided Breast Cancer Now with a rich portrait of women's lives and experiences across a range of areas including the following:

- Stage in the breast cancer journey is critical to people's experiences, needs and motivations
- There are differing attitudinal typologies in how people deal with their breast cancer journey
- There are high and differing information needs at specific points of breast cancer journey
- At the end of active treatment women feel adrift and fear secondary breast cancer
- Women with a diagnosis of secondary breast cancer can feel neglected, as though no one cares
- Partners often feel at a loss of how they can help and feel excluded.

This research has provided Breast Cancer Now with valuable insight to guide their wider strategy and patient focused work.

P6.24

Breast Density and Impacts on Health

Cheryl Cruwys

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The World Health Organization states "Early detection in order to improve breast cancer outcome and survival remains the cornerstone of breast cancer control" [1]. Breast Density Matters UK is a non-profit breast cancer organization. The organization's mission is to educate about breast density and its screening and risk implications with the goal of achieving the earliest stage diagnosis possible for women with dense breasts. This educational mission is endorsed by breast imaging experts worldwide [2].

Keywords: breast cancer, breast density, dense breasts, mammography, ultrasound, early detection

Breast density has implications for both breast screening and risk [3]. Dense breast tissue both obscures cancers on a mammogram and is also an independent risk factor for the development of breast cancer. Whilst dense breasts are common and not abnormal, it is known that mammograms are less effective in dense breasts and supplemental screening can increase the detection of early-stage breast cancer in dense breasts. Cheryl Cruwys, co-founder of Breast Density Matters UK, was diagnosed with breast cancer in May 2016. Her breast screening was conducted in France and because she has dense breast tissue; she received a supplemental ultrasound. The ultrasound detected what the mammogram did not, an asymptomatic 8-mm invasive tumour. Found

early, her treatment was minimal with a positive health outcome. Incidences have been reported of women, with dense breasts, who have been diagnosed with later stage, more advanced cancers whilst having previously received 'normal' mammograms [4].

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A list of full references can be found here:

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CLINICAL LATE DISEASE

P7.1

Outcomes of Everolimus-Exemestane in ER positive, HER2 negative heavily pre-treated metastatic breast cancer patients: a real-life multicenter experience

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Background: Everolimus-Exemestane is well established therapeutic option for ER+ and HER2− locally advanced and metastatic breast cancer patients as shown by BOLERO-2 trial. This retrospective observational study was done to assess patterns outcomes in real-life setting of nonselected population outside the large standalone cancer centers.

Aim and methods: Retrospective data collected for 61 patients who received Everolimus-Exemestane for metastatic breast cancer, ER+ and HER2−, between May 2012 and July 2015 from electronic prescribing systems at Lancashire Teaching Hospitals and East Lancashire Hospitals. The patients had previous failure on hormone therapy ± failure on chemotherapy. Primary aim was to analyse progression-free survival (PFS), and secondary aims included assessing overall survival(OS), dose modifications, toxicities, treatment interruptions in a real-world setting.

Results: Mean age was 56 years (range 25–82). ECOG PS0 2%, PS1 49%, PS2 49%. Seventy percent patients had one line of therapy before Everolimus–Exemestane, for 30% patients it was third line, and in 7% as fourth line. Bone and lymph node metastasis were commonest at 79 and 46%, respectively. At the time of assessment, 12 patients were still on treatment.

Median PFS and OS were 0.45 years (95% CI 0.365–0.527) and 1.43 years (95% CI 0.882–1.977), respectively.

Reason to stop treatment was disease progression in 49% whilst it was toxicity in 18%. Stomatitis, fatigue, infections and rash were commonest toxicities. 29.5% patients had grade-3 toxicities. No grade-4 toxicity. 18% patients required dose reduction to 5 mg. 24.6% patients needed treatment interruptions.

Conclusion: Although our treatment population had metastatic disease, was unselected, and heavily pre-treated, the results of PFS, OS and toxicity were comparable to BOLERO-2 trial. We found higher incidence of stomatitis and non-infectious pneumonitis while lower incidence of rash and infections. Our study confirms that Everolimus-Exemestane remains an important option for heavily pre-treated metastatic breast cancer patients.

P7.2

Outcomes of central nervous system radiotherapy for metastatic breast cancer: the Royal Marsden experience 2000–2016 (Abstract previously accepted for presentation at SABCS 2017)

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Background: Breast cancer (BC) is one of the most common malignancies affecting women. Brain metastases (BM) are frequently seen in BC, and can have devastating consequences with significant associated morbidity and mortality. Whole brain radiotherapy (WBRT) is commonly used to treat BM, with variable use of stereotactic radiotherapy (SRT). This study reports on the outcomes of BC patients with BM who received central nervous system (CNS) radiotherapy over a 17-year period at the Royal Marsden Hospital (RMH).

Methods: We included all BC patients who had WBRT with or without SRT for intra-parenchymal BM secondary to BC at RMH between 2000 and 2016 inclusive. Instances of meningeal involvement were excluded from analysis. Data collected included age, histological subtype, tumor grade, stage at original BC presentation, receptor status, date of BM diagnosis, number of metastases, size of largest BM, Eastern Cooperative Oncology Group (ECOG) score, presence of extra-cranial metastases (ECM), neurosurgery (NS) and stereotactic radiotherapy (SRT) details, and date of last follow-up or death. Univariate and multivariate analyses were performed to analyze the effect of each variable on overall survival (OS) from date of BM.

Results: A total of 426 patients were included with a median age of 54 years at BM diagnosis and a median time to BM from BC diagnosis of 43 months. At diagnosis, 94% had invasive ductal carcinoma (IDC) and 70% had Grade 3 disease. Stage IV disease at original BC presentation was seen in 18% of patients. Estrogen receptor (ER +) was positive in 57% ($n = 236$), progesterone receptor (PR+) in 44% ($n = 147$), and HER2 (HER2+) in 44% ($n = 166$). Twenty-two percent ($n = 89$) were triple-negative (TN). Median number of BM was 4 (range 1–205) and 20% ($n = 72$) of patients had only 1 BM. Average size of the largest lesion was 26 mm (range 1–75). The ECOG score was 0–1 in 61% of patients. Ten percent of patients ($n = 44$) underwent SRT and 10% ($n = 43$) underwent NS. Three hundred and eighty patients had died at the time of analysis. Median OS from date of BM was 6.4 months. On univariate analysis, age < 60 years at BC diagnosis (8.1 vs. 4.0 months, $p = 0.0007$) and BM diagnosis (8.0 vs. 5.6 months, $p = 0.03$), ECOG status 0–1 (9.6 vs. 4.0 months, $p = < 0.0001$), ER+ (8.0 vs. 6.0 months, $p = 0.0007$), PR+ (7.6 vs. 6.9 months, $p = 0.04$), HER2+ (10.5 vs. 5.6 months, $p < 0$ 0.0001), SRT (20.3 vs. 5.9 months, $p < 0.0001$), and NS (20.3 vs. 6.2 months, $p < 0.0001$) significantly predicted for improved OS. Triple-negative status predicted for worse survival (5.6 months vs. 8.1 months, $p < 0.0001$). On multivariate analysis, ECOG status, ER+, HER2+, treatment with SRT, and NS were independent predictors for OS.

Conclusions: This study confirms substantial heterogeneity of prognosis in patients with BM from BC, with significantly improved survival in patients selected for SRT or surgery. Further studies are required to optimize the role of CNS radiotherapy techniques such as SRT and hippocampal sparing WBRT in patients with a favorable prognosis.

P7.3

Spatial heterogeneity of response on whole-body MRI to first line hormonal therapy predicts progression-free survival in metastatic breast cancer

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Background: Whole-body magnetic resonance imaging (WB-MRI) reliably identifies types of response to systemic therapy in metastatic breast cancer (MBC) through analysis of changes in water diffusivity, cellularity and cell variability.

We adapted a novel methodology that uses WB-MRI to capture spatial response heterogeneity data via the METastasis Response Assessment Diagnostic System (MET-RADS).

This study evaluates whether the extent of spatial heterogeneity seen at initial response assessment is predictive of progression-free survival (PFS) in patients on first line hormonal therapy for MBC.

Methods: Patients on first line hormonal therapy for MBC who had undergone baseline and on-treatment response assessment WB-MRI scans were identified. All scans were performed using a published WB-MRI protocol. Patients with disease progression at their first WB-MRI response assessment were excluded from further analysis. Criteria for response assessment utilised the methodology described by MET-RADS. A Likert five-point Response Assessment Category (RAC) score (1 = response highly likely; 2 = response likely; 3 = no change; 4 = progression likely; 5 = progression highly likely) was applied separately to 14 defined anatomic regions. Within each region, two RAC scores were recorded to reflect both the dominant and the next most common pattern of response behaviour. Data were therefore captured on inter- and intra-regional response heterogeneity.

A novel Response Heterogeneity Index (RHI) was calculated from the regional RAC scores. The RHI value summarised the overall response heterogeneity seen across all involved regions, with higher scores indicative of greater heterogeneity of response. Depth of overall response to treatment was defined as the mean RAC score for all involved regions, with lower scores indicative of a greater depth of treatment response. RHI and mean RAC score data were separated by median values into two groups for analysis.

Results: Thirty-three patients were suitable for analysis. Patients with higher levels of response heterogeneity (defined as RHI ≥ 4; $n = 16$) had significantly shorter PFS than those with RHI < 4 ($n = 17$; median PFS: 12 versus 24 months; log rank test $p = 0.006$). Depth of initial response, as defined by mean RAC score, and PFS were unrelated (mean RAC ≤ 2.5 ($n = 17$) versus mean RAC > 2.5 ($n = 16$); median PFS: 27 versus 23 months; log rank test $p = 0.699$). There was no correlation between RHI score and mean RAC ($r^2 = 0.002$).

Conclusions: Lower variability of response behaviour at first treatment assessment is predictive of longer treatment duration in patients receiving first line hormonal therapy for MBC. The spatial heterogeneity of response evident on WB-MRI may represent the genetic heterogeneity and clonal evolution of MBC, which are key factors in the development of treatment resistance.

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P7.4

Patterns of disease progression in patients with local and metastatic breast cancer as evaluated by whole-body MRI

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Background: Clinical evaluation of palpable breast or chest wall disease is important in the assessment of response to systemic therapy in patients with combined local and metastatic breast cancer. However, there is a lack of data describing the patterns of progressive disease (PD) in relation to local and metastatic sites in patients on systemic anti-cancer therapy (SACT).

Whole-body magnetic resonance imaging (WB-MRI) provides detailed information about the extent and distribution of local and metastatic disease in breast cancer. Recent published data have shown the superiority of WB-MRI over body CT scans at identifying sites of disease and therapy response. This retrospective study is designed to analyse the patterns of PD on WB-MRI, in patients on first line SACT for combined local and metastatic breast cancer.

Methods: Patients with stage IV disease at first diagnosis, or those previously treated for early breast cancer with local (breast or chest wall) and metastatic recurrence, were included. Patients were eligible for analysis if they had a baseline WB-MRI prior to starting first line SACT for metastatic disease, and subsequent WB-MRIs for response assessment up to the point of PD and a change in SACT.

Patient information and SACT data were collected from contemporaneous medical records. Data on sites of disease and sites of progression were collected from the original WB-MRI reports. All WB-MRI scans were performed using a published WB-MRI protocol.

Results: Thirty-one patients were suitable for analysis. Eighteen had metastatic disease at first presentation of breast cancer. Thirteen had both local and metastatic disease recurrence, eight after previous mastectomy and five after previous breast-conserving surgery.

Mean age of patients at metastatic diagnosis was 58.3 years (range 31–86 years). Fifteen patients received first-line chemotherapy, ten with Her2-targeted therapies. Sixteen received first-line hormonal therapy.

None of the patients progressed first in local disease and/or regional nodes only. Seven patients (22.6%) had evidence of first PD in their loco-regional disease along with concurrent PD at metastatic sites. Twenty-four patients (77.4%) had evidence of PD at metastatic sites without progression of loco-regional disease.

Of the 26 patients with bone metastases, 20 had PD of bone disease. Two patients without bone disease at baseline had new bone lesions evident at first PD.

Of the eight patients with liver metastases, four had progression of liver disease. Five patients without liver disease at baseline had new liver lesions evident at first PD.

None of the patients had a change in SACT performed for clinical disease progression only.

Conclusions: PD identified by WB-MRI occurs most commonly at metastatic sites rather than at palpable sites of loco-regional disease. Therefore, evidence of ongoing clinical benefit of SACT at loco-regional sites of breast cancer on clinical examination may give false reassurance about the disease status at metastatic sites. Regular imaging reassessments are advised for patients undergoing SACT for loco-regional and metastatic disease.

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P7.5

Defining the metastatic breast cancer (mBC) patient population within the Sussex Cancer Network

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The Sussex Cancer Network provides cancer care services to 1.2 million people along the south coast. This project aims to better understand disease presentation, treatment patterns and outcomes in the local mBC population.
Methods: Clinical coding was used to identify patients referred to the mBC multidisciplinary team (MDT). Clinical notes, laboratory, radiology and chemotherapy e-prescribing records were used to collect demographic, histopathological, staging, treatment and survival data.
Results: Data from 503 patients referred to the MDT until 01/08/2017 were included in data analysis. 121 and 168 new diagnoses of mBC were made in 2015 and 2016 respectively. The average age of patients referred was 60.9 years. 59.1% of all patients referred to the MDT remain alive at the time of analysis. 79.5% of patients were previously treated for early breast cancer. The remainder 20.5% presented with a de-novo presentation of mBC. Radiological imaging demonstrated that 43.1% of patients presented with a solitary organ site of metastases, most commonly bone metastases (46.34%) followed by lung metastases (17%). In patients with widespread metastases, bone remained the most common site of disease spread (34.7%) followed by lung (22.9%) and liver (15.41%) metastases. Majority of the patient cohort was hormone receptor positive (ER+ 80.8%; PR+ 59.4%). 21.9% of patients were HER2 positive. 45% of patients received hormone therapy [Letrozole in majority cases (60.6%)] and 43% received chemotherapy [Paclitaxel and Capecitabine in majority cases (each 21%)] as first line treatment. 19% of patients were treated with HER2 directed therapies. Survival data based on hormone and HER2 receptor status demonstrate a mean overall survival within each sub-group of 51.8 months (ER+ PR+ HER2−); 40.4 months (ER+ PR+ HER2+); 28.5 months (ER− PR− HER2−) and 31.1 months (ER− PR− HER2+). These data will lay groundwork for future analysis of factors that guide decisions towards onward lines of chemotherapy in treating mBC.
This project is partially funded by and developed in collaboration as part of a joint working agreement with Roche Products Limited

P7.6

Pertuzumab, Trastuzumab and docetaxel use in patients with Her 2 positive metastatic breast cancer—experience from Barts Health NHS Trust

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Background: Current evidence suggests that patients with HER-2 positive metastatic breast cancer should be treated with a combination of taxane chemotherapy with pertuzumab and traztuzumab (TPH). Pertuzumab is available via the cancer drugs fund. The aim of the study was to assess the outcomes with this regimen as a clinical audit.
Methods: This was a single-centre observational retrospective-prospective study. Patients with metastatic breast cancer commencing TPH between February 2014 and December 2016 were included. Data were collected from electronic patient records and independently verified. Progression-Free Survival (PFS) and Overall Survival (OS) were analysed using Kaplan–Meier Survival methods.
Results: A total of 48 patients (47 female, one male) were included with a median age of 54 years (range 35–81 years). Eight patients were > 70 years old. 65% ($n = 31$) of patients had visceral disease. 33% ($n = 16$) of patients had ER-positive disease and 33% were from the black ethnic minorities group.
The estimated median PFS was 18 months. Estimated median OS was 45 months. The median time for follow-up was 18 months (range 1–45 months). 27 patients discontinued TPH regimen due to toxicity ($n = 2$), progression ($n = 24$), and decline in cardiac function ($n = 1$). Five patients had treatment suspended temporarily due to decline in cardiac function. In cases where TPH was discontinued, 20 (74%) patients received second-line treatment. Eighteen patients (90%) were given trastuzumab ematansine (TDM-1) in line with the current NICE guidance. The reasons for not receiving second line therapy were patient choice or clinical deterioration. To date, 65% of patients are alive ($n = 31$).
Conclusion: The outcomes of the clinical audit were comparable to the CLEOPATRA trial (Baselga J, et al. N Engl J Med. 2012;366:109–119). In a majority of cases, TPH was stopped due to disease progression and appropriate second line therapy was offered.

P7.7

Metastatic Breast Cancer: Prevalence and Outcomes in the Leicester Cancer Centre

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Background: It is difficult to assess the impact of metastatic breast cancer on Oncology services as there are few robust databases recording these numbers. Patients with metastatic breast cancer (mBC) are living longer with more lines of treatment available. Data here will guide future service provision and measure outcomes to national standards.
Methods: University Hospitals of Leicester Cancer Centre appointed our first mBC cancer nurse specialist (CNS) in 2014. Data collected are on all patients interacting with the CNS over 24 months.
Results: There were 200 patients, 198 were female and two male. Those aged 40 and under accounted for 15%, 50 and over 60.5% and 70 and over 19%. Germ line mutations were present in 4.5%. The time lapsed from primary diagnosis of breast cancer to metastatic disease ranged from 2 months to 40 years with the mean being 100 months.

	n	%
De Novo	48	24%
Site of metastases		
Bone only metastases	37	18.50%
Visceral metastases	163	81.50%
Receptor status		
ER+ HER2−	123	61.50%
ER+ HER2+	26	13%
ER− HER2+	16	8%
ER− HER2−	18	9%
Unknown	17	8.5%
Survival outcome		
Those still alive:		
Bone only metastases	31	84%
Visceral metastases	94	58%

	n	%
De Novo	33	69%
Median follow-up	18.9 months	
Alive patients	20.6 months	
Deceased	8.6 months	
Mean time to recurrence		
ER positive, HER2 negative	100 months	
ER positive, HER2 positive	101 months	
ER negative, HER2 negative	65 months	
ER negative, HER2 positive	29 months	

Conclusion: Early data suggest that most fields are consistent with published data. There is an excess of de novo mBC at 24%, national figures would suggest $< 20\%$. This is likely biased by data collection from Medical Oncology practice. Median survival has not been reached for any subgroup and therefore could not be determined. With further analyses, these data will help determine number of lines of treatment for mBC patients, allowing prediction of oncology services provision at a multidisciplinary level and allowing outcomes to be compared nationally.

TRIALS IN PROGRESS

P8.1

OPTIMA: a prospective randomized trial to validate the predictive utility and cost-effectiveness of gene expression test-directed breast cancer chemotherapy decisions

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Background: Multi-parameter gene expression assays (MPAs) are widely used to estimate individual patient residual risk in ER-positive HER2-negative node-negative early breast cancer, allowing patients with low risk to safely avoid chemotherapy. Evidence supporting MPA use in node-positive breast cancer is limited. OPTIMA (Optimal Personalised Treatment of early breast cancer usIng Multi-parameter Analysis) aims to validate MPA's as predictors of chemotherapy sensitivity in a largely node-positive breast cancer population.
Methods: OPTIMA is a partially blinded multi-center, phase 3 randomized controlled trial with an adaptive two-stage design. The main eligibility criteria are women or men aged 40 or older with resected ER-positive, HER2-negative breast cancer and up to 9 involved axillary lymph nodes. Randomization is to standard management (chemotherapy and endocrine therapy) or to MPA-directed treatment. Those with a "high-risk" tumor MPA score receive standard management whilst those at "low-risk" are treated with endocrine therapy alone. The feasibility phase (OPTIMA prelim) evaluated the performance of several MPAs to select a test to be used in the main efficacy trial based on economic analysis, and assessed the feasibility and acceptability of a large UK trial. OPTIMA prelim used Oncotype DX as the primary discriminator; the main trial will use Prosigna (PAM50) with Prosigna Score ≤ 60 defined as "low-risk." The co-primary outcomes are (1) Invasive Disease-Free Survival (IDFS) and (2) cost-effectiveness of test-directed therapy. Secondary outcomes include IDFS in "low-risk" patients, quality of life and additional survival measures. An integrated qualitative recruitment study will identify and address challenges to recruitment and informed consent. Tumor blocks from all consenting participants will be banked allowing the performance of alternative MPA technologies to be evaluated. Recruitment of 4500 patients will permit demonstration of 3% non-inferiority of test-directed treatment with 5% significance and 85% power, assuming 3 years of follow-up and a control arm 5 years of IDFS of at least 85%. The addition of patients from OPTIMA prelim will allow non-inferiority to be assessed with 2.5% significance.
Results: OPTIMA-prelim recruited 412 patients in 23 months from 35 sites with a 47% acceptance rate. The main study opened in January 2017. Early progress indicates that the recruitment target is achievable in the intended 46 months timescale through the participation of > 100 sites
Conclusion: OPTIMA, as one of the two large-scale prospective trials validating the use of test-guided chemotherapy decisions in node-positive early breast cancer, is expected to have a global impact on breast cancer treatment. Experience from OPTIMA prelim showed that patient advocate support and close engagement with sites will aid trial success.
Funding: The project is funded by the UK NIHR HTA Programme (10/34/501). The views expressed by the authors are not those of the HTA Programme, NIHR, NHS or the DoH.
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P8.2

Study within a trial using a stepped wedge trial design: evaluating a decision aid on patients' decisional conflict in PRIMETIME

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Introduction: PRIMETIME is a prospective biomarker directed cohort study aiming to identify a group of breast cancer patients who can safely avoid adjuvant breast radiotherapy following breast conserving surgery. This group is deemed to be at such a low risk of local relapse that the potential benefits of radiotherapy are unlikely to outweigh the risks. The uncertainty patients face regarding healthcare decisions is known as 'decisional conflict'. Decision aids are interventions which help patients to weigh up the risks and benefits of treatments. Evidence suggests that decision aids reduce decisional conflict. The study within a trial (SWAT) concept enables trialists to conduct research embedded within a larger trial in an economic and efficient manner. This SWAT is designed to investigate the effect of a decision aid on patients' decisional conflict within PRIMETIME.
Proposed Method: The PRIMETIME SWAT will utilise a cluster stepped wedge trial design. The decision aid will be in video format. Decisional conflict will be assessed using a validated decisional conflict scale in clusters prior to and following implementation of the decision aid. All clusters will receive the standard patient information sheets and be randomised to receiving the decision aid video at increasing intervals from when their centre began recruiting to PRIMETIME using minimisation. The primary outcome is the reduction in decisional conflict.
Discussion: The cluster stepped wedge trial design ensures that by the end of the study all centres will have use of the decision aid as opposed to a parallel design which may be considered less favourable as some clusters would never introduce the decision aid. If we are able to determine that decision aids reduce decisional conflict, this would provide evidence to support increasing resources into the development of decision aids.

P8.3

MAMMO-50: Mammographic surveillance in breast cancer patients over 50 years of age

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Introduction: There is a lack of evidence or consensus amongst surgeons on optimum frequency or duration of follow-up including mammography for breast cancer patients aged 50 years and older at diagnosis. Mammo-50 aims to provide sound cost–benefit evidence whilst investigating alternative methods of follow-up.
Methods: A multi-centre, randomised controlled, phase III trial of annual mammography versus 2 yearly for conservation surgery and 3 yearly for mastectomy patients. There was a successful integrated internal feasibility study aiming to set up at least 100 actively recruiting centres by month 24 and/or recruit 1400 patients as well as a sister cohort to explore reasons for non-participation. The trial aims to randomise 5000 patients and incorporates quality of life (QoL), qualitative and sample collection sub-studies.
Results: To date (25th September 2017) 3913 patients (123% ahead of target) have been randomised between the two arms from 114 sites open to recruitment and additional four in site set-up. The results of the feasibility phase showed that the study could recruit the required number of patients from the 100 centres. This is truly a multi-disciplinary trial with 53% randomised by the surgeons, 28% by the radiologists, 19% oncologists and 2% nurses.
Of patients randomised, 80% have undergone conservation, 87% have invasive disease, 83% aged 55–75 years, 83% ER+ve and 73% undergoing hormone therapy. Patients enter the trial due to altruism and the main reason for non-participation is that they do not wish to change their mammographic schedule.
Conclusions: Mammo-50 is an important trial which demonstrated that is it feasible, acceptable to patients and clinicians and will provide clinicians with valuable risk-adjusted information to guide their future practice.

P8.4

Neo-RT: Pre-operative breast intensity modulated radiotherapy in patients receiving neo-adjuvant hormonal treatment for breast cancer—a feasibility study

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Background: Intensity modulated radiotherapy (IMRT) targets tumour, whilst minimising dose to non-target tissue. Given pre-operatively, this may increase the chance of breast conservation in patients receiving neo-adjuvant endocrine therapy for early breast cancer with minimal side effects. Delivering neo-adjuvant IMRT also provides an ideal opportunity for translational research.
Aims
Neo-RT is a feasibility study designed to:

(1) test feasibility of neo-adjuvant breast IMRT and endocrine therapy in patients where radiotherapy may make breast conserving surgery easier;
(2) Explore indication of decrease in mastectomy rate, possible predictive immunohistochemical/molecular and imaging biomarkers of response, and evidence of modulation of immune infiltrate in tumours after radiotherapy.

Methods
Key eligibility
Women with invasive breast cancer who have planned treatment with neo-adjuvant endocrine therapy where radiotherapy may make breast conserving surgery easier.
Treatment and investigations
Three weeks of IMRT followed by 20 weeks of neo-adjuvant endocrine oral therapy (letrozole/tamoxifen) before surgery.

Blood samples and biopsies will be collected and paired with MRI/MRI-PET scans at baseline, during radiotherapy, endocrine and surgery. After surgery, patients will be followed-up, including radiotherapy late toxicity and PROMS, annually for 5 years.
Endpoints
Primary: proportion of patients successfully completing neo-adjuvant radiotherapy and endocrine therapy followed by breast surgery.

Secondary: peri/post-operative complications; mastectomy rates; volume of residual disease and response to treatment; acute and late normal tissue radiation-induced toxicity.

Exploratory: molecular/radiological biomarkers of response and radiation-induced immune modulation using serial blood samples and tumour biopsies paired with functional imaging before, during and after radiotherapy.
Sample size (Simon two-stage optimum design)
With a 5% significance level and 80% power, 43 patients (maximum) are required to detect a rate of $\leq 50\%$ versus $\geq 70\%$.
Study progress
Neo-RT study will be open in late 2017.
Impact on breast cancer research
A potential benefit to patients may be reduction in mastectomy rates. Results will inform the design of larger studies investigating predictive biomarkers of response to breast irradiation $\pm$ novel agents including immunotherapy, which may increase local control and survival.

P8.5

PRADA trial—Initial findings. (Primary Radiotherapy And Deep inferior epigastric perforator flAp reconstruction)

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Background: Historically need for post-mastectomy chest wall radiotherapy (PMRT) has deterred breast multidisciplinary teams from offering immediate breast reconstruction because of a perceived increase in operative complications and negative impact on aesthetic outcomes.
Aims: To evaluate the technical feasibility and safety of performing a mastectomy with immediate deep inferior epigastric perforator flap (DIEP) reconstruction shortly *after* chest wall radiotherapy.
Methodology: Eligible patients undergoing neo-adjuvant chemotherapy with a plan for subsequent mastectomy with immediate DIEP reconstruction and PMRT were recruited from Royal Marsden Hospital/Imperial, London. The primary outcome measure was the presence of open breast wound > 1 cm and requiring dressings 4 weeks after surgery. Evaluation completed at 90 days
Secondary outcome measures include comparison of volume and symmetry between the reconstructed and non-reconstructed breast using 3D-surface imaging, patient satisfaction measured using the BREAST-Q reconstruction module and applanation tonometry assessing breast compressibility. Translational research into tumour and normal tissue radiobiology/biomarkers of response is ongoing with serial blood samples, biopsies and imaging.
Results (September 2017): Between May 2016 and September 2017, 25/30 patients were recruited of whom 20 have completed RT and surgery. Mean age 48.2 years (range 31–62 years), body mass index = 26.9 (range 19–35.5) and mastectomy weight 643 g (range 398–1110 g). Within 90 days there were no unplanned returns to theatre or flap failures, two micro-vascular anastomoses required revisions at primary surgery, and there was one delayed wound healing.
Conclusion: In this small study of high-risk breast cancer patients, altering the sequence of radiotherapy and surgery did not have a deleterious impact on mastectomy skin flap necrosis or DIEP flap failure. Our results suggest that mastectomy and DIEP flap reconstruction *after* radiotherapy is technically feasible and safe across a wide range of 'real life' patients. Further 'linked' translational and qualitative studies are ongoing.
**ClinicalTrials.gov Identifier: NCT02771938*

P8.6

NOSTRA-Feasibility: A study to identify patients with residual cancer following multi-modality neoadjuvant treatment for HER2-positive, ER-negative early breast cancer using image-guided tumour bed biopsies

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Background: In the era before HER2-targeted therapy, omission of surgery after clinically (as opposed to pathologically) defined response to neoadjuvant chemotherapy (NAC) has been associated with a high rate of local recurrence but no apparent adverse impact on survival. Preliminary studies show that pre-surgical biopsies are feasible and can detect residual cancer (Francis et al. SABCS 2016). The false negative rate of tumour bed biopsies post NAC is not known and will be partly dependent on the pathological complete response (pCR) rate. Dual targeted anti-HER2 therapy results in pCR in up to 80% of HER2-positive (HER2+ve), ER-negative (ER−ve), progesterone receptor-negative (PR) breast cancer.
NOSTRA-Feasibility Study will assess how accurately the presence of residual cancer after NAC can be excluded in this population and determine whether a randomised study of surgery versus no surgery would be appropriate.
Key inclusion criteria: HER2 +ve, ER−ve, PR > 1 cm or node-positive early breast cancer visible on ultrasound.
Fit for chemotherapy, dual targeted anti-HER2 therapy and surgery.
Methods: Eligible patients will, after image-guided tumour marking with clips, receive an approved chemotherapy, trastuzumab and pertuzumab schedule. After completion of NAC but prior to surgical resection, multiple image-guided biopsies using protocol specified targeting will be performed to sample across the tumour bed. After surgery, the amount of residual disease (if not pCR) using Residual Cancer Burden scoring will be correlated with the detection of tumour in the pre-surgical biopsies. Blood and tissue samples will be collected for central review and translational research.
Primary end point: False negative tumour bed biopsy rate.
Statistics/sample size: Setting a minimum acceptable level of 10% for false negative tumour bed biopsy and using an estimate of 70% pCR rate. To exclude a worst case scenario of $\geq$ 10% false negative biopsy results with 95% confidence and allowing for potential drop out requires 150 patients.

P8.7

KORTUC phase I/II trial testing a novel radiation sensitiser in patients with locally advanced/recurrent breast cancer: preliminary results

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Aim: To test the safety & efficacy of 0.5% hydrogen peroxide in 1% sodium hyaluronate gel (KORTUC) delivered by intratumoural injection twice-weekly during external beam radiotherapy (RT) to

patients with locally advanced/recurrent breast cancer with or without associated distant metastases.
Background: Preclinical studies and initial non-randomised clinical trials in Japan have suggested a significant radiosensitising effect of KORTUC, with published complete response (CR) rates of 79–100% at up to 5 years follow-up in > 100 patients. In comparison, a contemporary Japanese cohort of 108 patients receiving systemic treatment and RT alone recorded a CR rate of 36%. A UK Phase 1 trial ($n = 12$) commenced at The Royal Marsden in January 2017 to confirm safety of this treatment.
Methods: Patients with primary breast cancer ≥ 3 cm were recruited. 36 Gy in 6 twice-weekly fractions or 49.5 Gy in 18 daily fractions were selected according to performance status. Twice-weekly intratumoural KORTUC injections were administered (1 h prior to RT) under ultrasound guidance, commencing in the second week of RT. 2.5–5 ml KORTUC was injected, depending on tumour size.
Results: 8/12 patients were recruited; 5/8 have completed treatment. 4 patients received 36 Gy/6# and 1 patient 49.5 Gy/18#. All received concurrent endocrine therapy. KORTUC was tolerated extremely well with 100% compliance. 3/5 patients experienced Grade 1 local tumour pain during injection not lasting more than 30 min. Skin toxicity (CTCAE v4.03) was scored at G0 in one, G2 in two, and G3 in two patients (treated with bolus). All resolved to < G3 by 4-week post-RT. 3-month ultrasound assessment demonstrated good partial response in 4 patients, and stable disease in 1 patient.
Conclusion: In our experience, KORTUC is a safe and well-tolerated treatment, with no additional toxicity compared to standard RT alone. Phase 1 is expected to complete in December 2017 and will be followed by a multicentre randomised Phase 2 trial comparing RT ± KORTUC.

P8.8

ROSCO: Response to Optimal Selection of neo-adjuvant Chemotherapy in Operable breast cancer: A randomised phase III, stratified CEP17 biomarker trial of neo-adjuvant FEC versus TC

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Background: Early breast cancer patients are frequently treated with anthracycline and taxane-based chemotherapy exposing patients to multiple toxicities. Abnormal duplication of the centromeric region of chromosome 17 (CEP17) and either Topoisomerase 2A (TOP2A) gene amplification or deletion are identified in meta-analysis as specific markers of anthracycline sensitivity (Bartlett et al. JCO 2015). ROSCO tests the utility of these markers to select anthracycline- or taxane-based chemotherapy in the neoadjuvant setting.
Key entry criteria: Centrally Confirmed CEP17 duplication and TOP2A status either marker abnormal (Ab) or both normal (N) primary tumour > 2cms or documented axillary node metastasis
Key exclusions: Grade 1/2 ER PR rich, HER2−. Cardiac disease.
Study treatment: Randomisation to four cycles of 5FU, Epirubicin, Cyclophosphamide (FEC) or four cycles of Docetaxel, Cyclophosphamide (TC) followed by surgery. Patients with residual cancer receive four cycles of the alternative chemotherapy arm. HER2-positive patients receive concurrent trastuzumab ± Pertuzumab. Patients with biopsy proven axillary node metastases undergo combined blue dye and radioisotope guided SLNB and ANC during breast surgery. Other adjuvant treatment is Institutional standard.
Primary endpoint: pathological complete response (pCR).
Key Secondary endpoints: response; breast conservation; patient reported outcomes; safety, tolerability and long term outcomes. The false negative rate of a negative SLNB compared to ANC will be reported.
Sample size/stratification: 1050 patients will be randomised in a 1:1 ratio stratified by nodal status, ER, HER2, and biomarker status (Ab vs. N)
Analysis: Primary analysis will assess the interaction between the treatment effect and CEP17/TOP2A status to determine if a differential treatment effect exists between N and Ab. Sample size is based on the ability to detect improvement in pCR in the Ab group from 21% in the TC group to 30% in the FEC group (90% power, 10% significance).
Trial progress: As of September 2017, 259 patients have been randomised into the Trial from 27 sites. Thirteen sites are in set up. The Trial remains open to new centres wishing to participate.

P8.9

Chemoprevention in BRCA1 mutation carriers (CIBRAC): a proof of concept clinical trial

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Introduction: Currently, risk management options for BRCA1 mutation carriers include risk-reducing mastectomies (RRM), and selective oestrogen receptor modulators (SERMs). However, the majority of BRCA1-associated tumours are oestrogen receptor negative (ER−ve). SERMs do not reduce the incidence of ER−ve tumours, and therefore are unlikely to be effective in this group of women. Oestrogen and its metabolites have been shown to cause DNA damage, resulting in genomic instability in BRCA1-deficient breast cells, an early hallmark of BRCA1-related cancers. Based on this, we hypothesise that oestrogen suppression in BRCA1 carriers may reduce DNA damage in breast tissue, and thereby potentially reducing breast cancer risk.
Trial design: The study is an interventional, non-randomised, crossover study without masking. The primary purpose is to assess the impact of these drugs on suppression of DNA damage.
Pre-menopausal BRCA1 mutation carriers will be recruited from the Family History Clinic at Belfast City Hospital. The proportion of women who receive information packs who progress to trial entry will be recorded, with target recruitment of twelve women to the trial. Participants will complete quality of life questionnaires, provide blood and urine samples and undergo baseline ultrasound-guided breast biopsy. Half of these women will receive a SERM (tamoxifen) for 3 months, whilst the other half receive oestrogen suppression therapy (goserelin and anastrozole), and then provide repeat questionnaires and samples (blood, urine and breast biopsy). Treatment

groups will cross over for a further 3 months of treatment, with a final set of questionnaires and samples taken. Compliance with treatment and adverse events will be monitored throughout the study.

Eligibility criteria

Inclusion criteria:

- Female
- Age $\geq$ 18 years
- Pre-menopausal
- Known pathogenic BRCA1 mutation
- Intact ovaries
- No previous breast/ovarian/other carcinoma
- No previous use of chemoprevention
- Willingness to use non-hormonal methods of contraception

Exclusion criteria:

- BRCA1 mutation of uncertain significance
- Contraindications to study drugs or breast biopsies
- Pregnancy or breastfeeding
- Inability to give informed consent
- Patient awaiting risk-reducing surgery

Specific aims

The primary objective is to assess the feasibility of treatments by measuring successful recruitment rates and compliance. The secondary objective is to establish tolerability of interventions through quality of life measurements and adverse event occurrence.

Exploratory objectives will assess the potential of treatments to reduce DNA damage through analysis of breast biopsies using comet assays and immunohistochemistry for 53BP1 and γH2AX (markers of DNA double-strand breaks). Oestrogen and metabolite levels will be measured in blood and urine samples using UPLC-MS/MS.

Statistical methods

Not applicable due to nature and size of pilot study

Present accrual: One participant

Target accrual: 12 participants

This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P8.10

Value of on-treatment transcriptomic biomarkers in sequentially sampled breast cancers receiving neoadjuvant chemotherapy for accurate outcome prediction

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Introduction: The neoadjuvant-treatment window can potentially provide valuable prognostic information due to early changes in expression profiles of breast cancers. Changes during endocrine treatment in ER+ tumours have been found to offer improved predictive capabilities for disease progression and response. This study aims to improve accuracy of response and outcome prediction over existing prognostic tests.

Methods: A cohort of 37 neoadjuvant chemotherapy primary breast cancer patients was obtained. Differential expression analysis was performed across response groups (nine Responders, 28 Non-Responders) as defined by PCR and over treatment time to identify expressed genes indicative for response status. These gene lists were used for pathway enrichment analysis to find common up/down-regulated pathways unique to the pathological complete responders and non-responders. Enriched pathways were used to identify genes of interest unique to responders/non-responders and to examine if changes in expression over time could be used to predict response. Results were validated in a similar contemporary dataset (I-SPY 1 TRIAL).

Results: Initial results show overexpression of a number of collagens, histones and several proliferation markers in the non-responders. Early down-regulation in proliferation is indicative of response in the ER+ treatment setting, and our results show a correlation between up-regulation of proliferation (CDK1, CENPF, MKI67) markers across treatment time and poorer treatment response. Pathway analysis highlighted elevated regulation ARHGEF, a RHOA up-regulator with poor response, and reduced levels in responders. Validation of the binary classifier was only 51% accurate; however, all 26 responders (reported by PCR) were correctly captured in the I-SPY dataset.

Conclusion: Identification of gene lists and pathways to differentiate response groups is useful for the improvement of prognostic markers. Changes in these markers from early time points (2 weeks to mid chemo) may be useful for identifying non-responsive patients earlier in the treatment cycle. Currently, on-treatment sampling can help to identify response status via proliferation at early time points. Work is continuing to develop and evaluate robust binary classifiers for response to neoadjuvant therapies.

P8.11

PIONEER- Pre-operative wIndOw study of letrozole plus Progesterone receptor agonist Megestrol Acetate versus letrozole aloNE in post-menopausal patients with OEstrogen Receptor-positive breast cancer

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Background: Recently published preclinical findings provide new insights into the functional 'cross-talk' between the oestrogen receptor (ER) and progesterone receptor (PR) in breast cancer (Mohammed et al., Nature, 2015). The addition of a PR-agonist to anti-oestrogens directly modifies ERa chromatin binding and transcriptional response in breast cancer cells, and is anti-proliferative in vitro and in vivo.

Megestrol acetate (MA), an off-patent semi-synthetic progesterone derivative, has been licensed for many years as a treatment for ER+ metastatic breast cancer. There is also good evidence for the effectiveness of low-dose MA as a supportive therapy to ameliorate endocrine therapy-related hot flushes.

Trial design: PIONEER is a three-arm, open label, multi-centre randomised phase II pre-surgical window trial evaluating effects of 15 days of preoperative therapy with Letrozole (LET), or LET plus MA 40 mg, or LET plus MA 160 mg in post-menopausal women

with newly diagnosed, ER+ HER2− invasive primary breast cancer. Patients are being recruited in Cambridge, with 6–7 other UK sites due to open in 2017/8.

3-arm randomisation

A	LET 2.5 mg OD
B	LET 2.5 mg + MA 40 mg OD
C	LET 2.5 mg + MA 160 mg OD

The primary endpoint is % change in proliferation between baseline and day 15 tumour biopsies, measured by Ki67 IHC assessment. Secondary endpoints include the following: expression of Aurora Kinase A, Caspase 3 and AR/PR/EMT markers by IHC; and safety endpoints. Exploratory endpoints include transcription factor mapping of ERa/PR (ChIP-seq) and identification of ERa-associated proteins (RIME) on paired fresh-frozen samples.

Patients are randomised in a 1:1.5:1.5 ratio for arms A:B:C. Based on results from previous trials, a mean 66% reduction in Ki67 is anticipated for LET alone (arm A), and a 77.5% reduction for combination arms B and C, based on preclinical data. A recruitment total of 189 patients is required.

Pioneer will help determine if there is value in conducting a follow-on adjuvant study investigating longer term benefit of combining an aromatase inhibitor with MA, and if so, at what dose (40 vs. 160 mg).

PRE-CLINICAL—NEW TREATMENTS

P9.1

Plasma G-CSF predicts poor response to zoledronic acid in breast cancer

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Background: The majority of breast cancer deaths occur due to distant spread of the disease, and bone is the most common site of metastasis. Bisphosphonates, e.g., zoledronic acid (ZA), are used to treat patients with osteolytic disease, including metastatic breast cancer. ZA has been demonstrated to reduce disseminated tumor cells (DTCs) and breast cancer recurrence, but not all patients see this benefit and it is not yet clear what predicts benefit from adjuvant ZA treatment. We have investigated the mechanism whereby ZA reduces breast cancer progression.
Findings: Using in vivo models, we established that ZA renders myeloid/osteoclast progenitor cells (M/OCPs) tumor-suppressive, primarily by directing their lineage potential. In instances where the M/OCP lineage potential was modulated via either systemic or tumor-derived granulocyte-colony stimulating factor (G-CSF), M/OCPs did not differentiate into tumor-suppressive populations but instead into mature osteoclasts. High G-CSF was sufficient to abrogate the tumor-suppressive effect of ZA. Furthermore, we determined that in a subset of plasma samples from the AZURE clinical trial (where stage II/III breast cancer patients were received standard systemic treatment with/without adjuvant ZA), patients who had plasma G-CSF levels > 23 pg/mL at time of randomization experienced a significant reduction in disease-free survival with adjuvant ZA.
Conclusions: Our data indicate that patients with higher baseline plasma G-CSF levels will not likely observe reduction in breast cancer recurrence with adjuvant ZA, and in fact may have worse prognosis with adjuvant ZA treatment as compared to control. Our study lays a foundation for understanding breast cancer patient responses to ZA and suggests that finding ways to capitalize on M/OCP function and differentiation potential would constitute novel therapeutic approaches to prevent or limit metastatic disease in the bone.

P9.2

Global knockdown of cellular kinases identifies monopolar spindle 1 (MPS1) as a novel modulator of endocrine and palbociclib resistance highlighting a new role for MPS1 inhibitors

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Background: Estrogen-receptor-positive (ER+) breast cancer (BC) accounts for over 75% of diagnosed cases. Despite treatment, a large proportion relapse with endocrine-resistant disease, making it one of the greatest challenges for BC research. Using a kinome siRNA library screen, we identified MPS1, required for recruitment of the spindle assembly checkpoint complex, as strongly associated with resistance to endocrine therapy and palbociclib. Until now, the target population for MPS1 inhibitors has focused on triple-negative BC. Our unexpected finding shows the potential efficacy of MPS1 inhibitors in ER + BC resistant to endocrine therapies and palbociclib.
Methods: ER+ BC cell lines (MCF7, SUM44, ZR75.1, HCC1428 and T47D) adapted to estrogen independent growth (LTED) or sequential resistance to fulvestrant or palbociclib (991R) were subjected to a siRNA screen targeting 709 kinases. Z-scores were used to identify the most robust candidates. Cell viability upon MPS1 inhibition with CCT289346 (MPS1i), currently in IND-enabling studies, was assessed 2D and 3D. The class effect was confirmed with other compounds targeting MPS1. Impact of MPS1i on ER colocalisation and ER-transactivation was assessed using confocal microscopy and reporter assays, respectively. Effect of MPS1i on chromosomal alignment and time spent in mitosis was established by time lapse and confocal microscopy. BrdU incorporation and cell cycle were assessed by FACS. PARP cleavage was used to measure apoptosis. Global gene expression analysis of *MPS1* was carried out in two independent neoadjuvant studies of aromatase inhibitor (AI)-treated patients.
Results: Kinome knockdown identified targets associated with the G2/M checkpoint as strongly implicated in the LTED phenotype. In particular, MPS1 was the top common hit in LTED and 991R cell lines. Increase in MPS1 was evident in MCF7-LTED at both the transcript and protein level. Notably, the MPS1i caused a significant reduction in viability of the majority of endocrine-resistant and 991R cell lines tested (IC50 ranging between 25 and 100 nM).
Upon inhibition of MPS1, cells demonstrated shorter time in mitosis, aberration of cell cycle and amplified mitotic errors, resulting in increased apoptosis.

To evaluate the clinical relevance of *MPS1* in ER+ BC treated with endocrine therapy, we interrogated publicly available datasets from patients treated with neoadjuvant AI therapy. In the anastrozole cohort, on-treatment gene expression of *MPS1* ($p < 0.0001$) was significantly associated with poor response to anastrozole based on a 2 weeks residual Ki67 score $< 10\%$. In the letrozole cohort, increased on-treatment expression of *MPS1* ($p = 0.0118$) was associated with poor response based of tumor shrinkage $\geq 50\%$.
Conclusion: This novel finding shows that MPS1 inhibitors are capable of inducing mitotic aberrations and apoptosis in ER+ BC models resistant to endocrine therapy and sequential resistance to palbociclib, providing a new therapeutic strategy.
This abstract has previously been accepted for presentation at SABCS 2017 and has been submitted to the UK IBCS without modification.

P9.3

A role for Focal Adhesion Kinase in regulation of Invasive Ductal Carcinoma Stem-like Cells

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Introduction: Emerging evidence suggests that Cancer Stem-like Cells (CSCs) initiate recurrence. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase known to be overexpressed in the triple-

negative phenotype and traditionally known for its role in metastasis and proliferation. Our group has shown that FAK expression correlates with poor prognosis, radio-resistance and CSC regulation in DCIS.
We hypothesise that FAK inhibition will reduce CSC activity and tumour growth in invasive triple-negative ductal carcinoma.
Methods: We tested the effect of 0.5 mM FAK inhibition using VS4718 and PF562271 alongside current adjuvant treatment on 5 cell lines across all molecular phenotypes and 27 patient samples on CSC activity, utilising the mammosphere assay. We used SiRNA transfection to confirm effect of FAK inhibition on CSC activity.
We evaluated the effect of 2 weeks of FAK inhibition (50 mg/kg BD, po) on a triple-negative patient-derived xenograft model (PDX), measuring the effects on tumour growth alongside the mammosphere assay.
Results: FAK inhibition reduced CSC activity and self-renewal measured using mammosphere formation in all cell lines with percentage reduction in CSC activity summarised in the table below:

	MDA-MB 231	SUM159	Triple-Negative patient samples ($n = 5$)	Triple-negative PDX	FAK SiRNA MDA-MB 231
Primary MFE	15%*	32%*	49%*	39%*	76%*
Mammosphere self-renewal	42% ns	53%*	n/a	n/a	64%*

*$p = <0.05$, Two-way anova with post hoc tukeys test. NS = not significant

Pharmacological FAK inhibition led to a 40% reduction in tumour growth at 3 weeks post treatment ($p = <0.001$, GEE analysis) in conjunction with a decrease in CSC activity as shown by a reduction in MFE shown above.
Conclusions: We have shown that FAK inhibition in cell lines, patient samples and in vivo reduces CSC activity in luminal and triple-negative breast cancer, and reduces tumour growth in the triple-negative setting. These data highlight how FAK inhibition may be used in a clinical trial to help improve patient outcome in triple-negative breast cancer.

P9.4

Anti-IL1B therapy and standard of care agents: a double edged-sword to halt breast cancer bone metastasis

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Breast cancer bone metastases is incurable and associates with poor prognosis in patients. After homing and colonising the bone, breast cancer cells remain dormant, until signals from the microenvironment stimulate proliferation of these disseminated cells to form overt metastases. We have recently identified interleukin 1B (IL-1B) as a potential marker for predicting breast cancer patients at increased risk for developing metastasis and established a role for IL-1 signalling in tumour cell dormancy in bone. We hypothesise that tumour-derived and microenvironment-dependent IL-1B play major roles in breast cancer metastasis and growth in bone.

Here, we report our findings on the role of IL-1B signalling in breast cancer bone metastasis. Using a murine model of spontaneous human breast cancer metastasis to human bone, we found that administration of the clinically available anti-IL-1B monoclonal antibody, Ilaris, or the clinically available recombinant form of the receptor antagonist, Anakinra, reduced bone metastasis (photons/sec mean values: 3.60E+06 Placebo, 4.83E+04 Anakinra, 6.01E+04 Ilaris). In line with this finding, IL-1B or IL-1R1 overexpression in human breast cancer cells resulted in enhanced tumour cell dissemination and growth in bone (12.5, 75 and 50% animals with tumour in bone in control, IL-1B and IL-1R-overexpressing cells, respectively). The use of standard of care agents and/or anti-resorptive drugs is a treatment strategy for patients affected by breast cancer. Here, we combine anti-IL1B treatment (Anakinra) with standard of care agent (Doxorubicin) and/or anti-resorptive agent (Zoledronic acid) in a syngeneic model of breast cancer metastasis. Our experiments show that the triple treatment significantly impairs breast cancer metastasis ($p = 0.0084$).

In conclusion, these data demonstrate that IL-1B/IL-1R1 signalling plays an important role in the formation of bone metastasis and inhibiting its activity pharmacologically alone or in combination with standard of care therapies has potential as a novel treatment for bone metastasis.

P9.5

E-cadherin/ROS1 inhibitor synthetic lethality in breast cancer

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The E-cadherin *(CDH1)* tumour suppressor gene encodes a calcium-dependent cell–cell adhesion glycoprotein, which has roles in maintaining cell polarity, differentiation, cell migration and survival. E-cadherin dysfunction is a feature common to many epithelial tumours, with the highest incidence occurring in diffuse gastric cancer (50%) and lobular breast cancer (63%) and can occur via *CDH1* mutation, deletion or epigenetic silencing. Although E-cadherin dysfunction is relatively common, precision medicine approaches that exploit this pathogenic alteration are not yet available.

Using genetic and small-molecule perturbation screens in breast tumour cells with CRISPR-Cas9 engineered *CDH1* mutations, we identified a synthetic lethal interaction between E-cadherin deficiency and inhibition of the tyrosine kinase ROS1. Using data from large-scale genetic screens in molecularly diverse breast tumour cell lines, we found that the E-cadherin/ROS1 synthetic lethality was not only robust in the face of considerable molecular heterogeneity but was also elicited with clinical ROS1 inhibitors including foretinib and

crizotinib. ROS1 inhibitors induced mitotic abnormalities and multinucleation in E-cadherin-defective cells, phenotypes that were associated with a defect in cytokinesis and aberrant p120-catenin phosphorylation and localisation. In vivo, ROS1 inhibitors produced profound anti-tumour effects in multiple, distinct, models of E-cadherin-defective breast cancer. These data therefore provide the preclinical rationale for assessing ROS1 inhibitors such as the licensed drug crizotinib in appropriately stratified patients.

P9.6

SFX-01 targets STAT3 signalling to inhibit stem-like cells in breast cancer patient-derived xenograft tumours

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Background: SFX-01 is a novel therapeutic comprising synthetic sulforaphane (SFN) stabilised within α-cyclodextrin. Breast cancer stem-like cells (CSCs) have been identified in all molecular subtypes and are likely drivers of breast cancer (BC) metastasis and treatment resistance. We established previously that CSC activity in ER+ BC represents a source of therapeutic resistance (Simões et al., Cell Reports, 2015).
Methods: We investigated SFX-01 effects on breast CSC activity using mammosphere formation efficiency (MFE) and aldehyde dehydrogenase (ALDH) activity—ALDEFLUOR assay—in patient samples and patient-derived xenograft (PDX) tumours. Cells from primary ($n = 12$) and metastatic ($n = 15$) samples were treated with SFX-01 or vehicle control. Using a 2- or 8-week in vivo treatment, early (HBCx34) and metastatic (BB3RC31) ER+ PDX tumours were treated with SFX-01 alone or in combination with tamoxifen (TAM) or fulvestrant (FULV). Tumours were dissociated and MFE and ALDH activity assessed.
Results: SFX-01 in vitro reduced MFE of both primary ($0.19\% \pm 0.02$ vs. control $0.52\% \pm 0.06$) and metastatic patient samples (0.43 ± 0.04 vs. control $0.93\% \pm 0.07$). TAM and FULV increased MFE and ALDH activity after 2 weeks of treatment in vivo, which was abrogated by combination with SFX-01; for example HBCx34 MFE with TAM alone: $0.81\% \pm 0.07$ versus TAM + SFX-01: $0.34\% \pm 0.02$ and ALDH + with TAM alone $10\% \pm 0.4$ versus TAM + SFX $4.2\% \pm 0.4$. TAM + SFX-01 suppressed tumour growth at 8 weeks versus TAM alone in HBCx34 but not BB3RC31. FULV treatment maintained tumour growth suppression at 8 weeks and no additive effect was seen with SFX-01, although MFE and ALDH activity were suppressed. Mechanistically, SFX-01 potently suppressed the increase observed in phospho-STAT3 after anti-estrogen treatments and we are currently investigating STAT3 signalling effectors through RNAseq data analysis.
Conclusions: Our data demonstrate the potential of SFX-01 for clinically meaningful improvements to endocrine therapy in ER + BC by reversing CSC- mediated resistance.

P9.7

Progesterone receptor antagonists inhibit cancer stem cell activity in vitro and in preclinical models of breast cancer

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Seventy-five percent of Breast cancers (BCs) express oestrogen receptor (ER) and progesterone receptor (PgR), and such patients receive anti-estrogens such as tamoxifen as endocrine therapy. However, resistance to therapies results in tumour recurrence, as we have recently reported that cancer stem cells (CSCs) play a role (Simoes et al., Cell Reports, 2015).

Progesterone receptor is established to regulate normal breast epithelial stem cells, suggesting PgR as a potential therapeutic target in breast CSCs. To investigate the effects on breast CSCs, we tested 2 non-steroidal PRAs; onapristone (Arno Therapeutics.) and AZ4425 (AstraZeneca), in ER/PR+ BC cell lines (MCF-7 & T47D) and 24 patient-derived samples (PDS) comprising early and metastatic BC. CSC activity measured with the mammosphere (MS) suspension assay in vitro, the limiting dilution assay in vivo, and anti-tumour potential in a ER+/PR+ patient-derived xenograft (PDX) model. In addition, new predictive biomarkers such as PgR accumulation into nuclear foci (PgRNF) were investigated to facilitate personalisation of anti-progestin therapies.

In vitro AZ4425 (100 nM) inhibited mammosphere forming efficiency (MFE) in MCF-7 cells by 32% ($p < 0.0001$) and in T47D by 21% ($p < 0.0001$). Onapristone produced similar results of 34% inhibition in MCF-7 ($p < 0.002$) and 34% in T47D cells ($p < 0.0002$). AZ4425 treatment significantly reduced MFEs in 6/10 early BC PDS and in 9/14 metastatic PDS. Onapristone also produced comparable reductions in MFEs, 5/10 in early and 7/14 in late BC PDS, respectively. In vivo transplantation of T47D cells under an AZ4425 72-hrs pre-treatment reduced tumour formation and CSC frequency. A BC-PDX model treated for 24 days showed greater anti-tumour potential in combination with tamoxifen than with AZ4425 alone. However, MFEs were significantly reduced with AZ4425 alone or in combination. Pilot data on PgRNF as a biomarker suggest larger responses to PgR antagonists in PgRNF + tumours.

In conclusion, combination of anti-progestins with current anti-oestrogen therapies such as Tamoxifen may overcome resistance mechanisms by targeting breast CSCs that are resistant to endocrine therapy.

P9.8

Towards in vitro oncology trials: drug testing in breast patient-derived organoid cultures

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Triple-negative breast cancers (TNBCs) lack targeted therapies. In part, the limited success for development of therapies for the TNBC subtype can be attributed to a lack of effective pre-clinical models. It is widely recognised that PDX models are gold-standard in vivo models of breast cancer. However, there is a need for disease-relevant in vitro models with the capacity to recapitulate the biology of patient tumours. This would also reduce and refine the number of animal experiments required for pre-clinical drug testing. Current in vitro models using clonal cell lines for high-throughput studies lack pathophysiological relevance to patients, thus limiting their capacity to predict tumour responses to therapies.

Three-dimensional (3D) organoid systems provide an additional level of complexity in vitro. Such systems have been shown to be more biologically relevant with increased cell–cell interactions for a wide range of tumour types. However, robust protocols are limited for breast cancer subtypes, including TNBC. We have developed in vitro TNBC models suitable for high-throughput screening. Using cells derived from our defined panel of patient-derived xenograft (PDX) tumours, we have generated a cohort of patient-derived organoid (PDO) models to recapitulate the complexity of tumours. To date, we have developed PDOs from both PARP inhibitor-sensitive and -resistant PDX tissue. We have also generated PDO models directly from patient chemotherapy-resistant patient tissue and current work is focusing on the validation of a chemotherapy and TDM-1 resistant, HER2+ PDO model. Using a high-content microconfocal imaging system and phenotypic profiling, we have tested sensitivities of organoid lines to standard of care treatments.

The combination of high-throughput phenotypic screening and patient-derived 3D models will allow us to rapidly test hypothesis-driven drug/target combinations, and establish whether organoid readouts can predict clinical responses and ultimately inform new treatment regimes and therapies for the benefit of TNBC patients.

P9.9

Epigenetic therapy as a potential combination treatment for breast cancer

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Extensive research has demonstrated the existence of aberrant epigenetic patterns in breast cancer cells, contributing to tumorigenesis. Reprogramming the epigenome of tumour cells using epigenetic therapies such as DNA methyltransferase (DNMT) inhibitors is a promising therapeutic strategy, providing novel therapeutic options. Some FDA-approved drugs (decitabine; azacitidine) are already in clinical use for the treatment of haematological malignancies. Nevertheless, in the case of solid tumours, epigenetic therapies have not received approval so far, failing to demonstrate sufficient efficacy and tolerable toxicity. Recently, vitamin C has been described to act as a cofactor of ten-eleven translocation (TET) enzymes involved in active DNA demethylation and to increase response to DNMT inhibitors in cancer cells.

With this in mind, this study aims to (i) evaluate the combined use of vitamin C to improve response to DNMT inhibitors in in vitro models of breast cancer and (ii) assess the potential of combination treatments for sensitising breast tumour cells to subsequent immunotherapy.

In some breast tumour cell lines grown as monolayers, an increase in cell death was observed when decitabine was combined with vitamin C compared to treatment with decitabine alone, confirming that vitamin C enhances decitabine effects in vitro. Epigenetic effects were associated with double-stranded RNA up-regulation and high induction of type I interferon pathway, characteristic of an increased viral mimicry previously reported to be induced by decitabine. In parallel, PD-L1, a target of immune-checkpoint therapy, and NY-ESO-1, a highly immunogenic cancer testis antigen, were up-regulated. Our results suggest that initial treatment with demethylating agents may be of potential benefit prior to immunotherapy for breast cancer. Further work is on-going, evaluating the effects of epigenetic drug treatment effects on 3D in vitro models of breast cancer. Future investigations will help understand the benefit of epigenetic therapy for sensitising tumour cells to immunotherapy in vivo.

PRE-CLINICAL—BIOLOGICAL STUDIES

P10.1

Regulation of breast cancer tumorigenesis, metastasis and osteolysis by IkappaB kinase epsilon

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The NFκB pathway plays an important role in inflammation and bone remodelling. IκB kinase subunit epsilon (IKKε), a key component of the NFκB signalling pathway, has recently been identified as a breast cancer oncogene but its role in the regulation of breast cancer metastases remains unknown. Here, we tested the effects of pharmacological inhibition and knockdown/overexpression of IKKε on mammary and skeletal tumour growth, metastasis and osteolysis in preclinical models of breast cancer. IKKε is highly expressed in various human and mouse breast cancer cell-lines and its stable knockdown in MDA-MB-231 cells or pharmacological inhibition with the IKKε/TBK-1 inhibitor, Amlexanox, suppressed cell migration, invasion (39%, and viability, whereas its overexpression was stimulatory. Moreover, Amlexanox (30 μM) significantly enhanced the anti-tumour effect of a panel of chemotherapeutic agents including Docetaxel by up to 38% ($p < 0.01$). In bone marrow cell culture, conditioned medium (10%v/v) from naive MDA-MB-231 cells enhanced RANKL-stimulated osteoclast formation (114%, $p < 0.001$), and this was abolished in cultures treated with Amlexanox (3 μM, $p < 0.001$) or exposed to conditioned medium from IKKε deficient MDA-MB-231 cells ($p < 0.001$). In vivo, administration of Amlexanox (35 mg/kg/day) in immuno-competent mice inoculated with mouse 4T1 cells reduced skeletal tumour burden (70%, $p < 0.01$) and protected against osteolysis (BV/TV, 69%; trabecular thickness, 51%; connectivity density, 120%; $p < 0.01$). Combined administration of Amlexanox (35 mg/kg/day) and Docetaxel (15 mg/kg/week) in mice reduced mammary tumour growth (59.7%, $p < 0.001$), improved survival (1.82-fold, $p < 0.005$) and reduced incidence of metastases to bone, brain and spleen ($p < 0.05$), when compared to vehicle or individual treatments. Collectively, our findings suggest that, due to the combined anti-tumour and anti-resorptive effects, IKKε inhibitors, alone and in combination with chemotherapeutic agents, show promise for the treatment of both skeletal and non-skeletal complications associated with advanced breast cancer.

P10.2

ZIP7-mediated zinc signalling drives anti-hormone resistance in breast cancer

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Zinc is one of the most abundant metals in the human body and its imbalance has been implicated in several types of cancers, including breast cancer. Zinc homeostasis is controlled by two zinc transporter families: the ZnT family (SLC30A) of zinc exporters and the ZIP family (SLC39A) of zinc importers. ZIP7 (SLC39A7), a ZIP family member, resides on the endoplasmic reticulum membrane and releases zinc from intracellular stores after activation by CK2 phosphorylation on S275 and S276. We have previously implicated ZIP7 in driving tamoxifen-resistant breast cancer using our model (TamR) which exhibits both increased ZIP7 expression and higher zinc content. Now, using our new pZIP7 antibody, which only recognises activated ZIP7, we show a significant fivefold increase in activated ZIP7 protein. This result holds true in models of both tamoxifen and Faslodex-resistant breast cancer. The immediate downstream targets of ZIP7-mediated zinc release are MAPK, PI3K/AKT and mTOR pathways, explaining its relevance to cancer. For the first time, we investigate pZIP7 in the Faslodex-resistant breast cancer model (FasR), which demonstrates more than a twofold increase in zinc, similar to that seen for TamR cells. This is coupled with a tenfold increased activation of AKT and MAP kinase, confirming our recent investigations. Interestingly, we have observed that although only 15% of basal MCF-7 cells are positive for pZIP7, tamoxifen-resistant cells are 100% positive for pZIP7, a fact that remains even during longer-term resistance. These data pave the way for new strategies to tackle breast cancer resistance such as using a CK2 inhibitor (CX-4945) to prevent the development of more aggressive phenotypes following an increased ZIP7 activation.

P10.3

KIAA0020/PUM3 is a triple-negative breast cancer dependency gene that functions in replication fork restart and repair

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Patients with Triple-Negative Breast Cancers (TNBC) generally have poor prognosis and lack targeted treatment options. Genomically, TNBCs have high levels of structural alterations and harbour scars of defects in homologous recombination. Genes located in these genomic regions represent potential therapeutic targets either by acting as drivers, or provide tumour-specific dependencies.

We identified copy number driven upregulation of *KIAA0020 (also referred to as PUM3) a* gene located in a TNBC recurrent amplicon on 9p24. Knockdown of KIAA0020 impaired the growth of a subset of breast cancer but not mammary epithelial cell lines. Additionally, the growth defects observed upon KIAA0020 knockdown in SUM149, a *BRCA1* deficient cell line, were reversed by reactivation of *BRCA1*'s function.

Phenotypically, KIAA0020 knockdown led to an increase in gH2Ax and 53BP1 nuclear foci, impaired restart of stalled replication forks, and decreased RAD51 mediated repair of collapsed replication forks. As a result KIAA0020 depleted cells have reduced DNA replication rate and slowed cell cycle progression. Furthermore, KIAA0020 knockdown increased the sensitivity of breast cancer cells to the replication stalling agent hydroxyurea and the PARP-inhibitors olaparib and talazoparib. The latter two of which induce replication fork stalling through their 'PARP trapping' activities. Knockdown of KIAA0020 also impaired replication stress induced PARylation and PAR foci formation and KIAA0020 and PARP1 knockdown showed phenotypic epistasis with respect to cell growth providing a potential mechanism for KIAA0020 s replication fork related functions.

By studying KIAA0020, we have identified a novel player in replication fork restart and repair whose activity may be particularly relevant in homologous recombination deficient TNBCs. A greater understanding of KIAA0020's function and the pathways that regulate it may identify novel therapeutic targets for TNBCs. Importantly,

direct or indirect modulation of KIAA0020's functions may potentiate the effects of replication stress inducing therapies such as PARP-inhibitors.

P10.4

SOX11 promotes invasive growth and ductal carcinoma in situ progression

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We show that SOX11, an embryonic mammary marker that is normally silent in postnatal breast cells, is expressed in many oestrogen receptor-negative preinvasive ductal carcinoma in situ (DCIS) lesions. Mature mammary epithelial cells engineered to express SOX11 showed alterations in progenitor cell populations, including an expanded basal-like population with increased aldehyde dehydrogenase (ALDH) activity, and increased mammosphere-forming capacity. DCIS.com cells engineered to express SOX11 showed increased ALDH activity, which is a feature of cancer stem cells. The CD44+/CD24−/ALDH+ cell population was increased in DCIS.com cells that expressed SOX11. Upregulating SOX11 expression in DCIS.com cells led to increased invasive growth both in vitro and when they were injected intraductally in a mouse model of DCIS that recapitulates human disease. Invasive lesions formed sooner and tumour growth was augmented in vivo, suggesting that SOX11 contributes to the progression of DCIS to invasive breast cancer. We identified potential downstream effectors of SOX11 during both microinvasive and invasive tumour growth stages, including several with established links to regulation of progenitor cell function and prenatal developmental growth. Our findings suggest that SOX11 is a potential biomarker for DCIS lesions containing cells harbouring distinct biological features that are likely to progress to invasive breast cancer.

P10.5

Electrophysiological characterisation of ion channel activity in tumour slices

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Voltage-gated sodium channels (VGSCs) are up-regulated in breast cancer compared to normal breast tissue, and their expression correlates with a worse prognosis. Blocking VGSCs with ranolazine or phenytoin, or knocking down the VGSC pore-forming subunit $Na_v1.5$, reduces tumour growth and metastasis in mouse xenografts. Also, upregulating the VGSC β1 subunit increases tumour growth in mice. The mechanisms by which VGSCs promote metastasis are not yet understood. Cancer cells have a relatively depolarised membrane potential (V_m) compared to normal cells, and V_m can control cell cycle progression and cellular migration. We hypothesise that VGSCs functionally contribute to breast cancer progression by regulating the V_m. We generated tumours by implanting GFP-expressing metastatic MDA-MB-231 cells in the mammary fat pad of $gc^{-/-}rag2^{-/-}$ mice. We took tissue slices from primary tumours and lungs of these mice for electrophysiological recording. We also made slices from human tumour samples from the Breast Cancer Now Tissue Bank. We used whole cell patch clamp recording to measure the ionic currents and V_m from cells within these slices. We found that sodium currents were similar in primary tumours and lung metastases from the mouse model, and that larger sodium currents did not appreciably change the V_m. We compared control MDA-MB-231 xenograft tumours with those in which the VGSC β1 subunit was upregulated. β1 increases the sodium currents of MDA-MB-231 cells in vitro, so we hypothesised that we would find a similar effect in mouse xenografts. However, we found no difference in sodium currents or V_m between the control and β1-overexpressing tumours. Human tissue slices are more difficult to record from than xenograft slices as they are less cellular and of more heterogeneous composition. So far, we have detected outward (likely K^+) currents in two human tumours. Our data show that it is possible to take electrophysiological recordings from mouse and human breast tumours to assess ion channel function.

P10.6

Tamoxifen: an apparent up-regulator of metastasis-inducing protein, the anterior gradient protein 2, in breast cancer

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Objective: Anterior gradient protein 2 (AGR2) has been shown to hold prognostic value, being predictive of poor survival in oestrogen receptor positive (ER+) breast cancers. High expression of AGR2 has been linked with poor response to tamoxifen, and use of tamoxifen has been suggested to increase the expression of AGR2. Our pilot study investigated the association between the level of AGR2 expression and treatment with tamoxifen in adjuvant and neoadjuvant therapy, compared to surgery and tamoxifen alone and the relationship between AGR2 expression and disease recurrence.
Design: Breast carcinoma specimens from women in three patient cohorts [adjuvant tamoxifen therapy with recurrence ($n = 19$); primary endocrine therapy ($n = 5$); neoadjuvant therapy ($n = 11$)] were immunohistochemically stained using a polyclonal antibody against AGR2 and AGR2 expression was compared to expression in archival data of women treated with mastectomy alone ($n = 166$) in which AGR2 was associated with recurrence. A cut-off of 25% distinguished low/no from moderate/high level of staining for AGR2.
Results: ER+ tumours of patients who underwent adjuvant therapy with recurrence were found to be significantly associated with a higher level of AGR2 staining, when compared to treatment with surgery alone (Fishers Exact Test; $p < 0.0001$), whereas non-recurrence was associated with lower level of staining ($p < 0.0001$). Neoadjuvant endocrine therapy was also associated with a higher level of AGR2 staining (54.5% of 11 specimens), when compared to treatment with surgery alone ($p < 0.02$).
Conclusion: We have shown that breast cancer recurrence and endocrine therapy is associated with higher AGR2 expression when compared to surgery alone in our patient cohort. AGR2 expression may be a biomarker of breast cancer recurrence for patients given endocrine therapy.

P10.7

Molecular mechanisms of therapy resistance in oestrogen receptor positive breast cancer

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Chemotherapy is a common component of treatment for breast cancer; however, chemotherapy resistance, whether intrinsic or acquired, can lead to disease recurrences and death. Our aim is to identify the molecular pathways driving resistance to chemotherapy in breast cancer. We have identified a cohort of oestrogen-receptor-positive (ER+) breast cancer patients, treated with neoadjuvant chemotherapy (epirubicin/cyclophosphamide), who displayed a partial response to therapy. We have investigated gene expression patterns in matched pre- and post-chemotherapy samples. Our hypothesis is that gene expression changes in tumour cells that survive chemotherapy are associated with therapy resistance.

Epithelial breast cancer cells were isolated from matched pre- and post-chemotherapy tumour samples from five patients using laser-capture microdissection. MicroRNA expression was determined using qPCR microarrays (360 microRNAs) and mRNA expression using NanoString profiling (770 mRNAs). Genes that consistently differed in expression post-therapy across all five patients were identified. Three microRNAs were selected for further analysis based on fold-changes: miR-26b, miR-195 and miR-10a (mean fold-changes: 4.9, 2.6 and 0.54, respectively). These microRNAs were tested for roles in defining chemosensitivity individually and in combinations by transfecting microRNA mimics and inhibitors into ER+ cell lines and assessing responses to epirubicin using cell viability assays and colony-forming assays. Up-regulation of miR-26b and miR-195 resulted in increased chemoresistance, in accordance with their up-regulation post-treatment in clinical samples, whereas miR-10a down-regulation resulted in increased chemosensitivity.

MicroRNAs influence cellular function by regulating the expression of their mRNA target genes. *CCDC6* is a predicted target of miR-26b and miR-195 and is down-regulated when breast cancer cells are transfected with mimics of these microRNAs. However, although *CCDC6* was a plausible effector of the chemoresistance phenotype, it does not appear to contribute directly to chemoresistance in this case. Identification of the functional effectors of these microRNAs is ongoing.

P10.8

The role of fibroblasts in chemoresistance in triple negative breast cancer

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Triple-negative breast cancer (TNBC) accounts for 10–15% of breast cancers and is associated with relatively poor prognosis. TNBC is characterised by lack of ER, PR and Her2 expression, and consequently does not respond to targeted treatments routinely used to treat other breast cancers. Therefore, cytotoxic chemotherapy is the only systemic treatment for TNBC. Resistance to chemotherapy in TNBC is a major clinical issue and is reflected in the high rate of early recurrences. Carcinoma-associated fibroblasts (CAFs) are often the most abundant cell type in the tumour stroma and have been shown to influence behaviour of the tumour cells. Our aims are to determine whether CAFs modify responses of TNBC cells to chemotherapy, to identify molecular pathways responsible for this cross-talk, and to develop these pathways as potential therapeutic targets to enhance chemotherapy responses.

We have shown that breast CAFs, but not normal fibroblasts, dose-dependently protect the claudin-low TNBC cell lines MDA-MB-231 and MDA-MB-157 from the chemotherapeutic epirubicin, in both short-term and colony-forming assays. By contrast, the basal claudin-high TNBC line MDA-MB-468 was not protected, demonstrating that the CAFs' influence is specific to certain TNBC characteristics. Protection was also provided by CAF-conditioned medium, showing that secreted factors are involved. Transcriptome profiling demonstrated that CAF-induced protection of MDA-MB-231 cells was associated with deregulation of specific genes and pathways, including activation of the interferon-signalling pathway. Modulation of interferon-signalling activity using recombinant interferons, blocking antibodies, or under-/over-expression of specific signalling intermediates modified chemotherapy response of MDA-MB-231 cells. Preliminary data suggest treatment with antibodies that block interferon signalling reduced CAF-induced protection, highlighting a potential therapeutic strategy to sensitise cancer cells to chemotherapy in stromal-rich tumours.

P10.9

The effects of stress hormone signalling on endocrine resistant breast cancer cells

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Endocrine resistance, either intrinsic or acquired, is attributed to the loss of ER expression in a proportion of patients. Faslodex (fulvestrant) can be considered during management of endocrine-resistant ER + breast cancers, but resistance to Faslodex can also develop, often accompanied by a loss of the ER. Increased expression of the glucocorticoid receptor (GR) in de novo ER-cells is associated with poor prognosis, and stimulation of the GR by glucocorticoids has been shown to promote oxidative stress and DNA damage.

Here, an acquired Faslodex-resistant cell line (FAS-R), characterised by a loss of ER, was generated by continuous antihormone exposure to the ER+ breast cancer cell line MCF-7. Both cell lines were exposed to the glucocorticoid, cortisol and expression of GR quantified using qRT-PCR. Release of reactive oxygen/nitrogen species (ROS/RNS) in response to cortisol was measured using electrochemical sensors, and the effects of cortisol on DNA damage/repair were measured using immunofluorescence and the comet assay. Expression of DNA damage sensors and repair effectors was examined using qRT-PCR.

Constitutive expression of GR was significantly higher in the ER-FAS-R cells compared to MCF-7 cells ($p < 0.001$). FAS-R cells expressed higher GR ($p < 0.05$) and significantly higher levels of ROS/RNS in response to cortisol, which were negated using mifepristone ($p < 0.001$). Cortisol also induced a significant increase in DNA damage and decreased capacity for repair in FAS-R cells compared to MCF-7 ($p < 0.05$). FAS-R cells expressed a higher basal and cortisol-induced level of DNA damage response sensing and repair proteins (e.g. p21, p53 and RAD51).

These data suggest the deregulation of DNA damage sensing and repair pathways through GR may act in a similar manner to de novo ER- disease. It is possible that patients with endocrine-resistant breast

cancer that lose ER at relapse should be administered glucocorticoids such as dexamethasone with caution.

P10.10

Phosphatidylinositol 3-kinase is a potential regulator of glutaminolysis in luminal B breast cancer

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Background: Altered cellular metabolism is a hallmark of cancer where some tumours become "addicted" to Glutamine (Gln) for their sustained proliferation and survival. Glutaminase (GLS) plays an important role in glutamine metabolism where it functions as a key enzyme in the Gln-Proline regulatory axis converting glutamine to glutamate. GLS is highly upregulated in Triple-Negative (TN) and HER2+ classes of breast cancer (BC) and targeted inhibitors are currently in Phase II clinical trials. We hypothesise that the mechanisms of GLS upregulation are variable in the different molecular subtypes of BC.
Methods: GLS expression was assessed in large, well-characterised BC cohorts at the DNA, mRNA (METABRIC, $n = 1980$) and protein level (Nottingham Series, $n = 1192$) and correlated with clinicopathological parameters, Myc and other regulatory proteins, and patients' outcome with consideration of molecular subtypes.
Results: High expression of GLS protein was positively associated with proliferative proteins (Ki67, Cyclin E, p16) and c-Myc expression ($p < 0.001$) but negatively associated with PIK3Ca ($p < 0.001$) and mTORC1 ($p = 0.014$). Within BC subtypes, GLS protein was positively associated with high PIK3Ca expression in only the ER+/HER2− High Proliferation (luminal B) subtype ($p = 0.008$). There was a positive relationship between GLS and mTORC1 in HER2+ tumours ($p = 0.003$) and high p53 expression in TN tumours ($p = 0.008$). Low GLS mRNA ($p = 0.049$) and protein ($p = 0.030$) expression predicted a worse survival in HER2+ tumours.
Conclusions: We provide comprehensive clinical data indicating that PIK3Ca is a potential regulator of glutaminolysis in the aggressive subclass of luminal BC. We propose using a variety of in vitro functional assays to validate our observational findings and assess their value as therapeutic targets in BC.

P10.11

A novel in vitro approach to determine the role of Hypoxia Inducible Factor 1-alpha in breast cancer

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Breast cancer is the most common cancer in the UK and once metastasised it is incurable making it the leading cause of death from the disease. Hypoxia within the tumour micro-environment greatly influences breast cancer progression and is linked to increased metastasis. Therapies blocking hypoxic signalling have, therefore, been developed but so far very little success has been achieved meaning more work is needed to further elucidate the role of hypoxia and its effects both locally and systemically.

Modelling hypoxia in a realistic way is difficult. In vitro, we generally use hypoxic incubators (1% O_2) or chemical mimetics ($CoCl_2$). These methods expose the whole population to hypoxia which does not represent reality; hypoxic areas are often small within a well-oxygenated tumour. This regional hypoxia may be important as it could influence cellular movement within or away from the tumour, cell death and therapy response as well as signalling in the local environment and at distant sites.

We developed a novel hypoxia-mimicking model in which HIF1-alpha can be induced in normoxic culture. This allows co-culture of HIF1-alpha expressing (hypoxic) cells with parental (normoxic) cells allowing examination of the interactions between the different populations.

Stable lines were produced expressing a mutant HIF1-alpha (stable in normoxia) fused to a destabilising domain. With the addition of Trimethoprim, the fusion becomes stable but without it is broken down rapidly. Induction of HIF1-alpha replicates the effect on cell signalling and the cancer stem cell (CSC) population seen in 1% oxygen/$CoCl_2$ culture. Conditioned medium taken following induction (as well as 1% O_2 culture) has the same effects, suggesting that secreted factors are responsible. When grown in co-culture significant changes in proliferation, gene expression and CSCs are seen within the parental and inducible cells suggesting bidirectional signalling between the populations.

This model will now be used to further study the effects of HIF1-alpha expression on other cell types in the local micro-environment, both cancerous and non-, and within the distant metastatic niche.

P10.12

Therapeutic resistance and stemness in mitotically quiescent human breast cancer cells

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Contemporary evidence supports that cancer stem cells (CSCs) are the metastasis-initiating population in breast cancer. However, isolation of CSCs based on specific marker signatures is hampered by population plasticity and the lack functional significance associated with this approach. We have established a reproducible in vitro model system allowing isolation of a breast cancer sub-population with a mitotically quiescent and stem-like phenotype that exclusively contains cells capable of escaping anti-neoplastic therapy to initiate new secondary colonies.

Cytofluorimetric monitoring of lipophilic fluorescent dye retention identified mitotically quiescent sub-clones ($\sim 0.05\%$) in monolayer cultures of five distinct human breast cancer cell lines (ER+ve and ER−ve). The quiescent population in both MDA-MB-231 and MCF-7 cell lines demonstrated significantly increased aldehyde dehydrogenase (ALDH) activity when compared to the bulk population and was enriched for $CD44^+CD24^+$ sub-clones.

Cytotoxicity assays with doxorubicin ($IC_{95} = 0.32$ μM) or paclitaxel ($IC_{95} = 20.80$ nM) demonstrated increased survival in the quiescent MDA-MB-231 fraction (40.76 ± 4.60 and $102 \pm 5.57\%$) compared with the bulk cell population (1.63 ± 0.22 and $4.56 \pm 0.25\%$, $p \leq 0.0001$). Similar assays with doxorubicin ($IC_{95} = 2.36$ μM) or paclitaxel ($IC_{95} = 46.45$ nM) in MCF-7 cells demonstrated increased survival in the quiescent fraction (22.68 ± 3.31 and $24.08 \pm 2.15\%$) compared with the bulk cell population (5.48 ± 0.92 and $6.34 \pm 1.01\%$, $p \leq 0.001$). Quiescent cells contained a sub-population ($\sim 1\%$) that was able to form new colonies following cessation of treatment. This sub-population was completely eliminated following the

pharmacological inhibition of oestrogen-related receptor-α (ERR-α) under nutritional stress.

We provide the first evidence that both MDA-MB-231 and MCF-7 human breast cancer lines contain a latent therapy-resistant quiescent cell population enriched with $CD44^+CD24^+$ and $ALDH^+$ sub-clones capable of outgrowth. Further characterisation of this prospective metastasis-initiating population might yield novel therapeutic targets for elimination of the cells responsible for breast cancer recurrence.

P10.13

Investigating the role of HORMAD1 in triple negative breast cancer

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HORMAD1, a gene normally only expressed in germline cells, was found present in 60% of Triple-Negative Breast Cancers (TNBCs). Our group showed that HORMAD1 expression is associated with higher levels of allelic imbalance in cancer genomes, which in turn correlate with response to platinum-based chemotherapy. In cells lines, HORMAD1 experimentally induces genomic instability through inhibition of Homologous Recombination (HR). Our aim is to identify genes modulated in response to HORMAD1 expression in mitotic cells, to provide further insights into HORMAD1's role in TNBC and reveal potentially targetable synthetic sensitivities. Inducible HORMAD1-expressing SUM159 clones were established and characterised with micronuclei assay, γH2AX and RAD51 focus-formation assay. Our clonal HORMAD1 inducible model recapitulated our prior findings with increase in aberrant nuclear structures and γH2AX foci, and decrease in RAD51 foci indicated a HR deficiency phenotype. Clones were then screened with an RNAi library targeting 1743 genes. Sensitising hits were validated in a deconvolution screen in the original clone, three additional HORMAD1-expressing and two GFP-expressing SUM159 clones. The siRNA screen identified 63 genes causing high sensitivities upon HORMAD1 expression and showed significant enrichment in DNA repair genes (14 out of 166, Hypergeometric test: $p < 0.01$). XRCC1, TDP1, POLH, BRIP1 and ATR were validated as having an on-target effect in three of four independent HORMAD1-expressing SUM159 clones compared to their parental isogenic control clones and GFP-expressing control SUM159 clones. Our preliminary data indicate that pathways preventing replication fork stalling or involved in its restart, i.e. base excision repair, DNA interstrand cross-link repair and translesion synthesis, are involved in the tolerance of HORMAD1 expression in somatic cells, as a result of HORMAD1-induced HR deficiency or other yet unknown mechanisms. These may reveal targetable dependencies relevant to a significant sub-population of basal-like breast cancer.

P10.14

Examining the role of IL6ST in endocrine resistance and trans-signalling in breast cancer

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At least seven cytokines share the ubiquitously expressed transmembrane receptor IL6ST (gp130) to mediate their actions. IL6ST has recently been identified as a predictive biomarker of the response to endocrine treatment, is associated with outcome in breast cancer patients, and is included in the 'Endopredict' test. Two signalling pathways for interleukin-6 have been identified: classic signalling (through the membrane-bound IL-6 receptor, IL-6R) and trans-signalling (via non-signalling membrane-bound soluble IL-6R, sIL-6R). Both pathways activate cells via IL6ST and may occur in parallel in cells expressing IL-6R and IL6ST. Trans-signalling plays a role in cancer and chronic inflammation and is inhibited by naturally occurring soluble forms of IL6ST, which can be detected in serum. Expression of IL6ST was assessed across three ERa+ breast cancer cell lines (MCF-7, T47D and ZR751) by western blot. Different concentrations of seven cytokines (IL-6, OSM, LIF, IL-11, CNTF, IL-27 and CT-1) were added to 10% FBS media to examine the effects on cell growth. The expression of full length IL6ST was observed in all three cell lines at different levels, with ZR751 and T47D having higher expression than MCF-7. Gel electrophoresis confirmed the presence of all previously described soluble forms in the three cell lines. Response to cytokines was variable, with OSM and IL6 stimulating growth of MCF-7, while cellular proliferation was inhibited in ZR751 and LIF stimulated growth in MCF-7 cells only. Perhaps surprisingly, no significant effect on growth was seen in T47D with any of the cytokines. A combination of sIL6R and IL6 significantly increased inhibition in ZR751 and stimulation in MCF-7. These results suggest that both classic and trans-signalling occur in two of three ER+ cell lines and gp130 could be a promising target for cancer therapy. Inhibiting IL6ST to determine cell growth after exposure to cytokines, oestrogen and tamoxifen will be examined.

P10.15

Regulation of voltage-gated sodium channel β1 subunit function and localisation by γ-secretase in metastatic breast cancer cells

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Voltage-gated Na^+ channels (VGSCs), composed of a pore-forming α-subunit and auxiliary β-subunits (β1-β4), are responsible for the inward Na^+ current underlying neuronal action potentials. VGSC expression is deregulated in cancer cells. β1 is upregulated in breast cancer specimens relative to control tissue and β1 overexpression in MDAMB231 breast cancer cells promotes tumour growth and metastasis and induces neurite-like outgrowths. β1-mediated neurite outgrowth in immature neurons is dependent on γ-secretase cleavage, which releases a β1 intracellular domain (ICD). Our study investigates the role of γ-secretase cleavage in β1 localisation and function in MDAMB231 cells. We hypothesised that β1ICD, the γ-secretase product, translocates to the nucleus, as seen with β2ICD, and that γ-secretase cleavage regulates β1 function. Imaged using confocal microscopy, β1ICD-GFP showed significant nuclear enrichment in MDAMB231 cells (MDAMB231-β1ICD-GFP), compared to full-length β1-GFP ($p < 0.0001$), but with similar enrichment to GFP ($p = 0.98$; one-way ANOVA). Other γ-secretase cleavage products like APPICD translocate as multimeric complexes. To determine whether β1ICD-GFP nuclear localisation was distinct from GFP nucleocytoplasmic diffusion, nuclear import was assessed using fluorescence recovery after photobleaching. The half-maximal recovery

time for β1ICD-GFP fluorescence in the nucleus was twofold greater than that observed for GFP ($p < 0.001$; Mann–Whitney test), suggesting a distinct nuclear import mechanism for β1ICD-GFP. Morphology and transcellular adhesion assays were used to assess the functional impact of γ-secretase cleavage. MDAMB231-β1ICD-GFP cells showed similar morphology in monoculture, evaluated by measuring process length, to MDAMB231-β1-GFP cells ($p = 0.09$), but almost double the process length of MDAMB231-GFP cells ($p < 0.01$; one-way ANOVA), suggesting β1ICD recapitulates aspects of β1-mediated cell morphology. Inhibiting γ-secretase in MDAMB231-β1-GFP cells significantly increased transcellular adhesion, but had no effect on control MDAMB231 cells ($p < 0.01$; t-test), implying that γ-secretase modulates β1-mediated cell–cell adhesion. Further work will focus on elucidating the role of nuclear β1ICD in MDAMB231 breast cancer cells.

P10.16

Regulation of 15-hydroxyprostaglandin dehydrogenase expression in breast cancer

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Cyclooxygenase-2 is over-expressed in several cancers, including breast cancer, leading to accumulation of oncogenic prostaglandin E_2 (PGE_2), associated with increased cellular proliferation, angiogenesis and metastasis. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the key PGE_2 metabolising enzyme and reduced 15-PGDH expression is observed in breast cancer. Consequently, up-regulation of 15-PGDH may be of therapeutic benefit in breast cancer. This project aims to assess the underlying mechanisms regulating 15-PGDH expression.

Expression of 15-PGDH in primary breast cancer was assessed by immunohistochemistry on tissue microarrays. Epigenetic silencing of 15-PGDH was studied through treatment of breast cell lines with demethylating agent, decitabine and histone deacetylase inhibitor, vorinostat. mRNA and protein levels were assessed using qPCR and immunocytochemistry. Pyrosequencing was performed to determine the methylation status before and after epigenetic drug treatment.

Results confirmed low 15-PDGH expression in breast cancer tissue with no link to tumour grade or receptor status. Some samples displayed high 15-PDGH staining in macrophages, which warrants further investigation. Breast cancer cell lines exhibit low 15-PGDH expression. Epigenetic drug treatment of MCF7 and MDA-MB-231 cell lines showed significant up-regulation of 15-PGDH mRNA and increased protein expression in the MDA-MB-231 cell line only. Preliminary pyrosequencing results indicate a low level of methylation at the 15-PGDH gene (*HPGD*) locus in the MCF7 cell line prior to drug treatment, which supports the lack of response to epigenetic drug treatment.

These findings indicate that 15-PGDH expression can be regulated by epigenetic modifications, but further research is required to determine whether increased 15-PGDH expression is directly or indirectly modified via epigenetic drug treatment. The interaction between macrophage and epithelial cells may be important in determining local tissue microenvironment prostaglandin levels. Further investigation is essential to confirm the role of epigenetic treatment in 15-PGDH expression.

P10.17

Progress towards the development of a fully humanised 3D cell culture model of HER2+ breast cancer

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HER2 is overexpressed in 15–30% of breast cancer and is currently treated with the monoclonal antibody Herceptin alongside chemotherapy. Due to resistance and cardiotoxicity in some patients, a therapeutic alternative to Herceptin is desirable. However, few oncology drugs make it through clinical trials, in part due to suboptimal modelling of human disease. Our aims are to create a fully humanised 3D cell culture model of HER2+ breast cancer, validate it as a drug testing platform using Herceptin and to investigate Affimers as a potential replacement to Herceptin. Affimers are a newly developed biosimilar that have advantages over monoclonal antibodies such as their small size, specificity and thermostability.

The BT-474 cell line was validated for HER2 expression and seeded into culture media that was free of animal-derivatives, epiFL media, an adaptation of commercially available FibroLife that was optimised for epithelial cell culture. SeedEZ, a hydrophilic scaffold of randomly arranged glass fibres, was used for 3D culture. The scaffold omits the need for an extracellular matrix such as Matrigel or collagen-I, both of which are derived from or contain animal components. To provide constant perfusion of media through the scaffold PerfusionPal was used. This has a reservoir that harbours a high-density blood-substitute that was infused and withdrawn to push media through SeedEZ, mimicking in vivo diffusion of nutrients from blood to tissue. Affimers that bind to HER2 were identified by phage display, expressed and purified from *E.coli.*

Cells grew successfully in SeedEZ ± perfusion. Perfusion did not influence the growth rate of cells but is expected to increase cell density. Less cell death was exhibited when cells were seeded in epiFL compared to regular media. Eight Affimers were identified and purified from *E.coli.* Going forward, Affimer ability to inhibit cell growth in PerfusionPal will be compared to gold-standard Herceptin. We anticipate our model to provide a 3D fully humanised preclinical drug testing platform that produces results consistent with what is observed in vivo, potentially increasing translation to the clinic.

P10.18

Modelling BRCA1-induced breast cancer with human induced pluripotent stem cells

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Germline mutations in the *BRCA1* gene predispose to early onset breast cancers, with a lifetime risk of up to 80%. Mutations in *BRCA1* have diverse effects on the BRCA1 protein, which is involved in various

cellular processes including DNA damage repair pathways. In addition to known pathogenic mutants, the *BRCA1* gene has many Variants of Unknown Significance (VUSs), leading to difficulties in clinical interpretation and prognosis. It is still unclear whether *BRCA1* acts as a tumour suppressor, or whether mutations are dominant-negative, causing functional disruptions which can be observed in heterozygous cells. There are inconsistencies between published studies on the impact of *BRCA1* variants on DNA damage response. Here, we aim to evaluate induced Pluripotent Stem (iPS) cells as a model to study the functional consequences of known pathogenic mutants and VUSs. Tissue samples have been collected from patients undergoing risk-reducing mastectomy or reduction mammoplasty. iPS cells were derived and characterised from fibroblasts of known BRCA1 pathogenic mutation (e.g. C61G) or VUS carriers, and non-mutant BRCA1 healthy controls. These lines have been characterised for pluripotency, and for any genomic instability due to reprogramming, by whole-exome sequencing. To evaluate the pathogenic effect of *BRCA1* mutations, different DNA damage repair assays have been carried out. Both known pathogenic and VUS lines presented a significantly higher number of chromosomal aberrations after γ-irradiation compared to healthy controls, together with obvious deficiencies in the Homologous Recombination (HR) repair pathway. Strikingly, damage levels, as assessed by measuring γH2AX foci formation, were significantly lower in known pathogenic lines than in VUS and control lines. Since *BRCA1* mutations cause breast cancers, assessment of mammary epithelial differentiation and tumorigenic potential of heterozygous mutants is being carried out. Taken together, our data suggest that DNA damage repair is impaired in iPS cells carrying *BRCA1* heterozygous mutations. This study also underlines the need for a wide range of assays to evaluate the impact of VUSs correctly.

P10.19

Anti-hormone induced trastuzumab resistance in breast cancer

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Prolonged anti-hormones (AH) are valuable in ER+ breast cancer, but many patients acquire resistance and relapse. Trastuzumab can be considered in the HER2+ cohort of these patients who previously received AH treatment, but there is only limited benefit. Here, we investigated whether the prior prolonged AH exposure might also have capacity to confer trastuzumab resistance by promoting alternative signalling in breast cancer.

Five acquired AH-resistant models generated by long-term (3 years) faslodex (FASR), tamoxifen (TAMR) or oestrogen-deprivation treatment of ER+/HER2+ lines BT474 and MDAMB361 were screened for trastuzumab impact. Affymetrix microarrays, PCR, Western blotting and immunocytochemistry explored erbB expression/localisation/activation, and downstream kinase activity in BT474FASR versus its time-matched control BT474(con) ± 7 days trastuzumab (100 nM). Transcriptome interrogation evaluated non-candidate pathways.

Three long-term antioestrogen-resistant models (MDATAMR/MDAFASR; BT474FASR) which had lost ER were also trastuzumab resistant, contrasting their ER+/HER2+ responsive parental lines. Focusing on BT474FASR, although this retained HER2, EGFR and downstream ERK1/2-MAPK signalling, there was decreased expression/activation of erbB3 ($p = 0.001$) and AKT ($p < 0.001$) with increased erbB4 ($p = 0.02$). Furthermore, trastuzumab failed to decrease HER2 expression, membrane localisation, or signalling through AKT in BT474FASR, contrasting significant inhibition in the trastuzumab responsive BT474 (con) line. Microarrays revealed non-candidate NOTCH signalling ($p = 0.005$) and stress-related (p38/JNK) MAPK pathway elements were also induced in BT474FASR.

Prolonged AHs are not only capable of inducing AH resistance and ER loss, but also trastuzumab insensitivity in ER+/HER2+ breast cancer cells. Deregulation of erbB signalling and loss of any ER cross-talk, as well as gains in non-candidate signalling, may underpin AH-induced trastuzumab-refractory growth. Our findings provide avenues for further investigation, including whether erbB4 or NOTCH targeting improves on trastuzumab impact in long-term AH-treated HER2+ cells.

P10.20

Increased ECM stiffness drives DNA damage and transformation of mammary epithelial cells through down regulation of aldehyde dehydrogenases

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Breast cancer is a leading cause of female morbidity and mortality. High mammographic density (MD), the proportion of the breast that appears opaque in a mammogram), is a principle risk factor for breast cancer development. Women with the highest quintile MD showing a risk 4-6 times that of those with the least dense breasts. How high MD promotes breast cancer is not understood, but recently it was established that high MD breast tissue is mechanically stiffer than low-density tissue. A number of studies have linked ECM stiffness with breast cancer invasion, but it is not known how a stiff microenvironment promotes transformation of normal mammary epithelial cells (MECs). To investigate this, we utilized a 3D alginate/matrigel culture model in which the mechanical stiffness could be varied to mimic that of normal breast tissue. Non-transformed MECs cultured within a stiff 3D-matrix acquired more DNA double strand breaks than those within a soft microenvironment. Cells within the stiffer 3D-matrix also showed increased transformation. To understand how ECM stiffness might promote genomic damage, we carried out RNASeq analysis to identify potential pathways. These data indicated that a number of signaling and metabolic pathways were altered in response to increasing ECM stiffness. In particular, the stiff microenvironment downregulated several isoforms of aldehyde dehydrogenase, which resulted in an increase in reactive aldehydes observed in MEC in stiff 3D-ECM. Expressing the aldehyde dehydrogenase Aldh3b2 using a stable lentivirus in cells grown in stiff environment reduced the levels of DNA damage and transformation to those seen in soft ECM. Together, this suggests that the stiff microenvironment associated with high MD breast tissue may contribute to an increase in reactive aldehydes and facilitate genomic damage and breast cancer risk.

P10.21

The effects of PTPN2 loss on cell signalling and clinical outcome in relation to breast cancer subtype

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Background: The protein tyrosine phosphatase PTPN2 is expressed throughout all tissues and thought to be a tumour suppressor. As PTPN2 is poorly studied in breast cancer, the aim of this study was to explore the role of PTPN2 in breast cancer.
Materials and methods: Protein expression and gene copy number of PTPN2 was analysed in a cohort of pre-menopausal breast cancer patients with immunohistochemistry and droplet digital PCR, respectively. Triple-negative cell lines MDA-MB-231 and MDA-MB-468, HER2-positive cell line SKBR3, and Luminal A cell line MCF7 were used for in vitro studies. PTPN2 was knocked down with siRNA transfection and cells were stimulated with HGF and EGF.
Results: Low PTPN2 protein expression was found in 50.2% of the tumours (110/219), gene copy loss in 15.4% (33/214). Low protein expression was associated with a higher relapse rate in patients with Luminal A and HER2-positive tumours, not triple-negative tumours. In vitro studies further revealed a subtype-specific role of PTPN2. Knockdown of PTPN2 had no effect on triple-negative cell lines, whilst knockdown in MCF7 inhibited phosphorylation of Met and promoted that of Akt. Knockdown in SKBR3 led to increased Met phosphorylation and decreased Erk phosphorylation as well as EGF-mediated STAT3 activation.
Conclusions: In conclusion, the role of PTPN2 in breast cancer seems subtype related. How much this affects treatment should be further investigated.

P10.22

Breast cancer metastasis to bone: the role of the perivascular niche in regulating tumour cell dormancy

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Tumour cell dissemination to the bone marrow is thought to be an early event, and disseminated tumour cells (DTCs) are found in the bone marrow of approximately 30% of breast cancer patients at the time they receive treatment for their primary tumour. However, only a proportion of these patients develop clinical symptoms of metastatic disease, often following an extended period of dormancy. The bone microenvironment plays an important role in regulating the dormancy of DTCs, but the cellular and molecular components of the bone metastatic niche remain to be clearly defined. We have used in vivo modelling to identify novel mechanisms regulating the dormancy of DTCs in bone.

We have established two mouse models of bone metastasis, where cancer cells arriving in bone either undergo outgrowth (6 weeks old animals with high bone turnover) or enter dormancy (12 weeks old animals with mature skeleton). We use flow cytometry and qPCR to quantify differences in both the cellular composition and gene expression signatures in these two models, with particular emphasis on the bone microvasculature and perivascular niche. We employed newly developed multi-colour confocal microscopy techniques to study and compare the micro-anatomical niches colonized by DTCs in these two models.

The bone microvasculature and associated perivascular niche are markedly different in these two models. We show that increased numbers of Thrombospondin-1^+ cells, vascular remodelling and reduced numbers of $CD31^+$ blood vessels, $Osterix^+$ and αSMA^+ osteoprogenitors, and $CD169^+$ macrophages are associated with the dormancy of DTCs in bone. In addition, we also show the differential expression of such dormancy-regulating genes as *Thrombospondin-1*, *Collagen-IV*, *Periostin*, *Tenascin-C* and *Osteopontin* between the outgrowth and dormancy-promoting niches in bone.

Our data indicate that tumour cell dormancy in bone is supported by the mature microvasculature, reduced bone turnover and alterations in the immune system.

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P10.23

The anion channel GPR89/GPHR finely regulates breast cancer protein folding homeostasis

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In order to sustain uncontrolled growth, breast cancer cells undergo significant stress due to accelerated metabolism, deficient nutrient provision and oxidative stress. The adaptive unfolded protein response (UPR) signalling is then activated to protect cells and to allow "normal" tumour growth by reducing protein synthesis and favouring chaperonine functions. This is achieved through modulation of the PERK-IRE1α-ATF6 signalling cascades. Although these genes activate pro-survival signals, they are also involved in apoptosis initiation but the mechanism controlling this balance is still undefined.

We have recently completed an integrated in silico/in vitro study aiming to identify novel tumour drivers and gene addictions in breast cancer. Analyses of gene copy-number alterations and gene-expression profiles in a Triple-Negative Breast Cancer-enriched cohort, identified the anion channel GPHR/GPR89, a driver gene required for breast cancer growth. Here, we show that GPR89 is amplified and highly expressed in our cohort and also in the publicly available TCGA-METABRIC datasets. Using an orthogonal characterisation, we demonstrate that GPR89 down-regulation impairs breast cancer cell growth and clonogenic ability. Furthermore, breast cancer spheroids inducibly overexpressing GPR89 are shown to have an increased area compared to control spheroids, demonstrating an important role of GPR89 in breast cancer cell growth.

UPR signatures based on first-degree protein–protein interactions indicate that GPR89 is positively correlated with IRE1α and negatively correlated with ATF4/CHOP networks. Consistently, GPR89 is also positively correlated with the XBP1 gene signature. Furthermore, by gradient sucrose subcellular fractionation and immunofluorescence analysis, we show that GPR89 has an ER localisation in breast cancer cells, where it modulates IRE1α/XBP1 and PERK/ATF4/CHOP signalling pathways and thus, suggesting to be a key regulator of breast cancer proteostasis.

In summary, we demonstrate that GPR89 is upregulated in breast cancer, where it controls protein homeostasis and, therefore, can be a novel target for breast cancer therapy.

P10.24

Effects of Platelet-derived growth factor-C (PDGF-C) signalling on extracellular matrix in breast cancer

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The PDGFs are important for activation of cells of mesenchymal origin such as fibroblasts and pericytes, both in normal and cancerous settings. In breast tumours, epithelial expression of PDGF-C has been associated with the basal subtype and a poorer outcome. We have previously shown that genetic ablation of *Pdgfc* in the MMTV-PyMT mouse model, as well as pharmacological inhibition of PDGF-C in xenografts, enabled treatment with tamoxifen in previously endocrine-resistant tumours. Hence, paracrine PDGF-C signalling involving tumour epithelial cells and fibroblasts is important for maintaining the basal phenotype in breast cancer. To determine what type of extracellular matrix (ECM) molecules signifies PDGF-C positive tumours, a mass spectrometry analysis of Pdgfrα (Platelet-derived growth factor receptor α)-expressing cancer-associated fibroblasts (CAFs) isolated from MMTV-PyMT $Pdgfc^{+/+}$ and $Pdgfc^{-/-}$ mice ($n = 4$) was performed. ECM-related proteins such as Col1a1, Col1a2, Col3a1, Fkbp10 and P3h3 were downregulated on average 25% in Pdgfrα-positive fibroblasts from MMTV-PyMT $Pdgfc^{-/-}$ mice compared to $Pdgfc^{+/+}$ mice. The ECM-related proteins were further validated in a human CAF cell line (CAF2) treated with PDGF-C for 6 and 48 h, respectively. Quantitative real-time PCR was used to determine expression of the ECM-related genes, anticipating an increase of transcripts. *COL1A1*, *COL1A2* and *COL3A1* were increased 1.7 to 4.2 fold after 48 h of PDGF-C stimulation, indicating a secondary response. To obtain an overall picture of differences in ECM-related gene expression in mammary tumours from MMTV-PyMT $Pdgfc^{+/+}$ and $Pdgfc^{-/-}$ mice, an RT2 Profiler PCR array (Qiagen) for mouse extracellular matrix and adhesion molecules was implemented ($n = 8$). Preliminary results indicate differences in transcripts such as the Adamts (A disintegrin and metalloproteinase with thrombospondin-like motifs) family of extracellular proteinases. Studies are ongoing to further characterise the impact of PDGF-C on breast cancer ECM, and how the ECM in turn affects the phenotype of tumour cells.

P10.25

'A Gremlin in the works: Reduced expression of bone morphogenetic protein antagonist Gremlin aids breast cancer progression'

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Background: Breast cancer is the most common cancer in females worldwide and despite advances in understanding disease progression and metastasis only 15% of patients diagnosed with metastatic breast cancer survive 5 years. Bone morphogenetic proteins (BMPs) are Transforming Growth Factor (TGF)β family members implicated in breast cancer progression and bone metastasis. However, little is known regarding the role of the secreted protein BMP antagonist Gremlin in this process.

Methods: Expression of Gremlin was examined in breast cancer cell lines using PCR. In vitro cell assays for growth and invasion used Gremlin overexpression plasmid and shRNA knockdown lentiviral vectors.

Gremlin expression in 82 breast carcinomas was compared to 24 normal mammary samples using qPCR and immunohistochemistry and correlated to patient clinicopathological parameters. Gremlin expression in clinical cohorts was examined using the gene expression omnibus (GEO) microarray database.

Results: Gremlin mRNA expression was lower in MCF-7 cells and higher in MDA MB 231 cells. Gremlin overexpression in MCF-7 cells reduced breast cancer cell growth ($p = 0.001$) and invasion ($p = 0.09$), whereas knockdown in MDA MB 231 cells significantly increased cellular growth ($p = 0.001$) and invasion ($p < 0.001$) compared to control.

Immunohistochemistry of breast carcinomas showed decreased Gremlin compared to normal mammary tissue. GEO data showed decreased Gremlin mRNA in carcinoma compared to normal tissue ($p = 0.05$). Our cohort had lower Gremlin expression in poor prognosis patients ($p = 0.048$), those with local recurrence ($p = 0.017$), or who died from their disease ($p = 0.016$). Expression was lower in those with bone metastasis ($p = 0.032$).

Conclusions: Lower Gremlin expression results in greater breast cancer growth and invasion in vitro. This correlates clinically to poor prognosis tumours. Understanding the influence of BMP antagonists could lead to developing therapeutics and biomarkers for disease dissemination or relapse. Our results suggest that Gremlin may have an important regulatory role in breast cancer progression and raises the question of whether this may have an impact on the metastatic process.

P10.26

Modelling myoepithelial dysfunction in ductal carcinoma in situ

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The transition of ductal carcinoma in situ (DCIS) to invasive breast cancer is characterised by the breakdown of the outer myoepithelial cell layer of the breast duct, allowing for the invasion of cancer cells into the extra-ductal tissue. The biology behind this transition is poorly understood but likely involves dysfunction in the myoepithelial cell, which adopts a more invasive phenotype in some cases of DCIS.

Using access to the Breast Cancer Now tissue bank, we have developed a 3D in vitro model of the human breast duct, using patient-derived myoepithelial and luminal cells as building blocks. This model accurately reflects the physiological bilayer of the breast duct and allows for the interactions between these two cell types to be interrogated.

Luminal expression of the oncogene *HER2* promotes a DCIS phenotype characterised by luminal filling. Combined myoepithelial expression of ITGB6, an integrin associated with high-risk DCIS, further disrupts the bilayer, promoting dramatic cellular invasion. This invasion is characterised by the collective invasion of luminal cells into the gel, with myoepithelial cells driving the invasive front. This myoepithelial-led invasion requires a permissive factor produced from luminal cells, since myoepithelial cells cultured in the absence of luminal counterparts fail to invade into gels, even following induction of ITGB6 expression.

We have demonstrated in a 3D model of the breast bilayer that myoepithelial cells can promote invasion. This model presents an excellent system to understand how myoepithelial cells promote

invasion, hopefully leading to new biomarkers to stratify management of patients with DCIS.

P10.27

Investigating the diversity of anti-estrogen resistant breast cancer stem cells

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Although considerable progress has been made in breast cancer research over the past few decades, more than 11,000 women died from this disease in 2014 in the UK. De novo or acquired resistance to standard anti-estrogen therapy is one of the main reasons for the high cancer mortality incidence in estrogen receptor-positive (ER+) breast tumours. There is evidence that Cancer Stem Cells (CSCs) are the culprits for the lack of response to anti-estrogen treatments. Therefore, this study sought to determine the effects of anti-estrogens on different CSC populations. We also aimed to characterise anti-estrogen-resistant CSCs and to investigate their cellular diversity, in order to identify biomarkers that can be therapeutically targeted.

The proportion of CSCs following anti-estrogen treatment in ER+ cell lines and patient-derived samples (PDS) was assessed using flow cytometry to measure cell surface antibody binding, autofluorescent cells and aldehyde dehydrogenase (ALDH) activity. The stem cell activity of ALDH positive cells was assayed using in vivo transplantation and in vitro mammosphere formation assays and their gene expression profile was studied using Affymetrix Arrays. Finally, ALDH pos cellular diversity was investigated at the single cell level using the C1 system and Biomark HD technologies (Fluidigm).

Our results show that ALDH-positive cells are enriched in ER+ cell lines and PDS following Tam and Fulv treatment. The ALDH1A3 isoform appears to be important at driving this enrichment in MCF-7 cells. ALDH-positive cells from MCF-7 and PDS have a distinct gene expression pattern when compared to ALDH-negative cells. Single cell analysis of the ALDH-positive population revealed that they are not a homogeneous cellular compartment and instead comprise 2 distinct clusters, which are present in different proportions before and after anti-estrogen treatment. Remarkably, a third cluster appeared only after Fulvestrant therapy.

In summary, anti-estrogen therapies enrich for cells with CSC properties, which comprise at least three distinct populations of cells. Future studies should assess the functional relevance of these cellular subgroups.

P10.28

Role of GPER in the angiocrine actions elicited by IGF1 in breast cancer

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The G protein estrogen receptor GPER mediates estrogen action in breast cancer cells as well as in breast cancer-associated fibroblasts (CAFs), which are key drivers of tumor progression. GPER cooperates with the Hypoxia Inducible Factor 1 alpha (HIF-1 alpha) toward the regulation of VEGF expression and angiogenesis in hypoxic breast tumor microenvironment. Furthermore, GPER contributes to the stimulatory actions elicited by the IGF1 signaling in breast cancer. Here, we evaluated the potential role elicited by GPER in the angiocrine actions of IGF1 in breast cancer. Analysis of 17 published Affymetrix microarray datasets of 2999 breast cancer patients and of Metabric studies performed on 1904 human breast tumor samples revealed that GPER is co-expressed with IGF1R and with the vessel marker CD34, suggesting its involvement in establishing an angiogenic signature. Next, we used GPER-positive but Estrogen Receptor-negative primary CAFs derived from breast tumors and SKBR3 breast cancer cells to investigate the role of GPER in the regulation of VEGF expression and angiogenesis by IGF1. RT-PCR, western blotting, immunofluorescence, and reporter assays allowed to determine that IGF1/IGF1R signaling activates the HIF-1α/VEGF pathway. A time-dependent increase of HIF-1α, GPER, and VEGF mRNA and protein expression was detected treating both SKBR3 cells and CAFs with 100 ng/mL IGF1. Reporter assays performed in SKBR3 cells showed that IGF1 triggers a nearly twofold increase in the luciferase activities of a HIF-1α, GPER, and VEGF reporter constructs. Biologically, the IGF1 increased endothelial tube formation by HUVECs in a HIF-1α- and GPER-dependent manner. These findings indicate that GPER serves as a crucial mediator in IGF1-dependent breast tumor angiogenesis. This suggests that targeting the interactions between cancer cells and the tumor microenvironment involving GPCRs and growth factor receptors has potential in future combination anti-angiogenic and anticancer therapies.

P10.29

NOTCH4–ERα crosstalk in breast cancer stem cells

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Many oestrogen receptor-positive (ERα+) breast cancers benefit from endocrine therapies, but 100% efficacy is limited by acquisition of endocrine resistance. Although endocrine therapies reduce tumour proliferation, they do not target a small subpopulation within the tumour called Cancer Stem Cells (CSCs) which have tumour-initiating ability. Breast CSCs do not express ERα but the activation of JAG1-NOTCH4 axis promotes their survival and self-renewal (Simões et al. 2015). In breast cancer, the importance of NOTCH4 and oestrogen signalling in promoting endocrine resistance remains unknown. Therefore, we aim to investigate the NOTCH4–ERα crosstalk in the regulation of breast CSCs. As experimental model, we generated NOTCH4 CRISPR KO MCF7 cells using the CRISPR-Cas9 technique. In the NOTCH4 CRISPR KO cells, we found a downregulation of other NOTCH receptors (NOTCH1, 2, 3), while no effects were observed on ligand expression (JAG1, JAG2, DLL1, DLL3, DLL4). Nonetheless, *HEY2*, a transcriptional repressor, was the only NOTCH target gene to be downregulated in the absence of NOTCH4. Interestingly, we detected a markedly increase in ERα expression at both protein and mRNA levels in NOTCH4-KO MCF7 cells compared to parental cells, corresponding to a higher activation of ER signalling. No difference was found in proliferation between

both cells lines. However, NOTCH4 CRISPR KO cells showed an important reduction in CSCs activity, using the mammosphere-forming and the ALDEFLUOR assays (1- vs. 0.5-fold, 1- vs. 0.46-fold parental vs. KO, respectively), and looking at $CD44^{+}/CD24^{-/low}$ population (1- vs. 0.37-fold parental vs KO). These results suggest that NOTCH4 signalling may regulate ERα and stemness. Understanding the mechanism that regulates this NOTCH4–ERα crosstalk could permit the development of new strategies to overcome endocrine therapy resistance and avoid recurrence.

P10.30

The Breast Cancer Now Tissue Bank: a rich resource to enable translational biomedical research in breast cancer

The Breast Cancer Now Tissue Bank

The Breast Cancer Now Tissue Bank, Leeds, UK

Introduction: The Breast Cancer Now Tissue Bank (BCNTB) was established in 2010 in response to a gap analysis identifying an urgent need for high-quality tissue samples for use in breast cancer research. The BCNTB comprises three core centres (University of Leeds, Bart's Cancer Institute, University of Nottingham) and two collaborating centres (University of Sheffield, University of Southampton) working together as one national resource, providing the UK's single largest collection of breast tissue samples.
Methods: Patients presenting at the centres above are consented under REC approval obtained separately for each institution and samples are stored under the authority of HTA licences at each of the core centres. Management of the bank and coordination of tissue requests is overseen by a team at BCN.
Results: Since its inception, the BCNTB has consented over 10,000 donors and collected more than 40,000 samples, in the form of frozen and FFPE tissue, blood, serum, plasma and buffy coat. Alongside the more common tumour types of all grades and receptor status, the collection includes substantial holdings of rare and clinically important samples including triple-negative tumours, rare malignant subtypes, ductal carcinoma in situ (DCIS), other benign tumour types and male breast cancer. The bank also has a large number of normal breast tissue samples, including normal surrounding tumour and those from risk reducing surgery and reduction mammoplasty. Pre-made and custom tissue microarrays (TMA), and longitudinal plasma samples suitable for cfDNA applications are also available. Furthermore, the BCNTB offers a bespoke primary cell culture programme (described in a companion abstract) and a data mining platform.
Conclusions: The BCNTB offers a source of readily accessible well-annotated patient samples, facilitating translational breast cancer research and welcomes applications from the research community to do this.

P10.31

Investigating the role of the tumour microenvironment in lobular breast cancer metastasis

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Invasive lobular carcinoma (ILC) accounts for 10–15% of diagnosed breast cancers, second to invasive ductal carcinoma (IDC), which accounts for 80%. ILC has an unusual pattern of metastatic dissemination, spreading to the gastro-intestinal tract, peritoneum and ovary. Despite prognosis and survival rates are originally favourable, patients with lobular histology appear to have a worse survival in multivariate analysis after a prolonged follow-up.

There is strong evidence supporting the importance of the tumour microenvironment (TME) in influencing tumour progression, metastatic spread and therapeutic response. However, little is known about the molecular mechanisms by which the TME influences the behaviour of ILC. Therefore, our research focuses on understanding how this may drive the unique features of ILC. We hypothesise that the interaction of ILC with the TME is critical for driving a disease distinct from IDC. In support of this, differential gene expression and proteomic analysis of ILC versus IDC tumour have revealed genes involved in cell adhesion, invasion, extracellular matrix (ECM) and actin cytoskeleton. One feature of the TME that may mediate some of these processes is cancer-associated fibroblasts (CAFs). We have collected CAFs from both ILC and IDC patients and have performed a transcriptomic analysis, where preliminary results have revealed differences in ECM-associated proteins between ILC and IDC-derived CAFs.

We have generated a valuable gene expression dataset comprising laser capture microdissection (LCM)-separated epithelium and stroma from 23 ILC samples obtained at time of surgery. Supervised analysis (Rank Products and SAM analysis) identified genes differentially expressed between the two cell compartments. As expected, our preliminary results outline clear differences in pathways related to the ECM, adhesion and cell cycle. Further analysis in the context of existing LCM-separated datasets of IDC will help us to elucidate how genetic changes within the TME affect ILC progression, metastasis and response to therapy.

P10.32

Investigation of progesterone and prolactin receptor signalling in the regulation of normal human breast stem/progenitor cell activity

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Progesterone and prolactin remodel mouse mammary morphology during the menstrual cycle and pregnancy by acting on the epithelial cell hierarchy including stem/progenitor cells. Local mediators such as RANKL, WNT4, STAT5 and Elf5 are co-ordinately regulated by progesterone and prolactin during mammary development. We aim to investigate whether progesterone and prolactin receptor signalling co-ordinately regulates stem/progenitor cell proliferation and differentiation in the normal human breast.

TMAs containing normal breast tissue were obtained from the Breast Cancer Now Tissue Bank. Samples were split into two categories: normal tissue, high risk of breast cancer ($n = 29$) and normal risk of breast cancer ($n = 42$). Immunohistochemical (IHC) analysis was performed for Estrogen Receptor (ER), Progesterone Receptor (PR) and Ki67. Matched frozen terminal ductal lobular unit (TDLU) microstructures were also obtained from the Breast Cancer Now Tissue Bank. Fluorescence-activated cell sorting (FACS) was performed ($n = 22$) to isolate four populations, mature luminal (ML), Luminal Progenitors (LP), Basal (B) and Stromal (S). RNA was extracted from the sorted populations of high risk ($n = 4$) and normal

risk ($n = 5$) patients and RT-PCR was performed on 23 genes known to define the different populations.

We detected a mean percentage positivity for IHC staining, ER (13.0; 27.2%), PR (10.2; 11.6%) and Ki67 (1.9; 4.0%) for normal-risk and high-risk samples, respectively. Statistical differences were found between normal- and high-risk groups for both ER and proliferation (Ki67). In the FACS sorted cells, we found expression of PR, ER, Prolactin Receptor (PRLR), WNT4 and STAT5A in the ML, RANK, KIT and ALDH1A3 in the LP and CD10 and VIM in the B cell populations. There were significant differences between the normal- and high-risk groups for PRLR, STAT5A, ER and ALHD1A3 gene expression.

In current studies, we are culturing TDLU microstructures with Progesterone and/or Prolactin and examining Ki67 and gene expression to help us understand how progesterone and prolactin co-ordinately regulate proliferation and differentiation in the normal human breast.

P10.33

Deciphering paclitaxel resistance in breast cancer

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Breast cancer is the most common form of cancer in the UK (Office for National Statistics, June 2016). While patients with hormone responsive breast cancers are typically treated with a regime of surgery and targeted therapies and experience generally positive outcomes, those with triple-negative or metastatic breast cancer experience a poorer prognosis. For these patients, treatment principally consists of cytotoxic chemotherapies, and although initial response to these drugs is usually positive, resistance will often develop and the decline of the patient soon follows. Resistance to chemotherapy therefore represents a significant clinical problem, but despite this the molecular basis for resistance is poorly understood, and limits the development of new strategies to overcome it.

We have recently identified that as cells enter mitosis, the pro-apoptotic Bcl-2 protein, Bid, becomes phosphorylated on S67, and leads to the induction of apoptosis should a cell remain in mitosis for too long. Anti-mitotic drugs, like paclitaxel, induce apoptosis through this mechanism; however, data indicate that the phosphorylation of Bid still occurs in paclitaxel-resistant cells, suggesting that they have acquired mechanisms to suppress Bid's function within mitosis. Here, we explore the use of proximity labelling enzymes to interrogate Bid's molecular interactions within mitosis, allowing us to reconstruct its role in inducing apoptosis during prolonged mitotic arrest and determine if it is possible to exploit this molecular pathway to resensitize cancer cells that are resistant to paclitaxel.

P10.34

Landscape of alternative splicing in breast cancer subtypes

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Breast cancer heterogeneity is driven by a complex system of interacting mechanisms. Differential exon usage (DEU), or alternative splicing is one such mechanism which facilitates the production of multiple protein isoforms from a single gene and extends the phenotypic capability of the genome. DEU has been implicated across many different cancer types; however, the landscape of DEU across different subtypes of breast cancer remains to be elucidated. We hypothesise that genomic aberrations are selecting for alternative splicing changes specific to breast cancer subtypes. To test this, here we catalogue alternatively spliced (AS) genes induced by somatic copy-number aberrations (SCNA) in oestrogen receptor-positive (ER+) and -negative (ER−) breast cancers as well as alternatively spliced genes independent of ER status. First, we selected genes with copy-number aberrations correlated with mRNA abundance (correlation $\rho > 0.3$, $q < 0.001$). Second, by prioritising top genes in each of these groups, we examined splicing changes in breast tumours stratified by genomic gains (SCNA $\log_2$ratio > 0.2). Using LIMMA diffSplice in the cancer genome atlas (TCGA) breast cancer dataset ($n = 1066$), we identified 283 AS genes in ER+, 10 AS genes in ER− and 208 AS genes independent of ER status (SIMES test $p < 0.05$). We note that majority of these changes ($\sim$60%) encompass alternative transcription start (TSS) or end sites (TES), with remaining changes at exons other than TSS and TES. We further assessed significant DEU events as candidate markers of disease recurrence in TCGA cohort and found a number of exons predictive of disease recurrence ($p < 0.05$). This large-scale exploratory analysis contributes a greater understanding of the complex landscape of alternative splicing events underling tumour heterogeneity and has highlighted several promising subtype-specific prognostic biomarkers which will be further validated.

P10.35

Metabolic reprogramming in endocrine therapy resistance in ER positive breast cancer

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The majority of breast tumours express oestrogen receptor (ER) and are dependent on oestrogen for their growth and survival. Endocrine therapy is the standard of care for this breast cancer subset and acts by targeting ER pathway. Despite its efficacy, a large proportion of women relapse with endocrine-resistant disease. By using a multidisciplinary approach of in silico, in vitro and in vivo assays, we are investigating the link between altered breast cancer metabolism and endocrine therapy resistance. We found that endocrine therapy-resistant cells can undergo a complex metabolic rewiring and are characterised by higher metabolic plasticity. In particular, we recently demonstrated the miR-155/hexokinase-2 axis to be an important regulator of the central carbon metabolic plasticity. In addition to central carbon metabolism, global transcription analysis of endocrine therapy resistant cells showed a deregulated node between miR-23b and the amino acid transporter SLC6A14 in endocrine therapy-resistant cells, which leads to an impairment of amino acids metabolism in the resistant cells with subsequent activation of autophagy. The miRNAs identified have both prognostic and predictive value in ER-positive breast cancer. These results suggest that a strategic adaptive mechanism characterised by high metabolic plasticity and independency from the extracellular environment is involved in acquiring adaptive features that allow breast cancer cell survival in the presence of endocrine therapy. Identifying the molecular mechanisms involved in this metabolic reprogramming may offer an array of potential targetable pathways to be exploited as monotherapies or

combinatorial approaches to combat or delay endocrine therapy resistance in ER-positive breast cancers.

P10.36

Overlap between osteoblastic and perivascular niches: fertile soil for breast cancer cells

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Bone metastasis is one of the most common complications of advanced breast cancer. During dissemination to bone, breast cancer cells locate in a putative 'metastatic niche' and this microenvironment plays a key role in the colonisation, maintenance of tumour cell dormancy and progression. The components of the niche include both osteoblasts and vessels that may represent therapeutic targets. We investigated the response to modification of the bone and perivascular niche on breast cancer cell homing.

Using either the bone-resorption inhibitor Zoledronic acid (ZOL) or the VEGF-receptor tyrosine kinase inhibitor Cediranib, we modified the metastatic niche in BALBc/nude mice. MDA-MB-231-NW1-Luc2 Vybrant-CM-DiI labelled breast cancer cells were injected i.c. to mimic the early steps of bone metastasis. Tumour cells in the long bones were detected in tibia using multiphoton microscopy, microvasculature visualised using immunofluorescence protocols against the endothelial marker Endomucin and quantified using Aperio ImageScope software. Changes in the bone structure were detected on histological sections of tibia following Toluidine Blue staining.

Breast cancer cells located preferentially in the trabecular region of metaphysis areas of the long bones compared to the growth plate area. This highly vascularized region is particularly rich in osteoblasts and it is the area where the drugs used in these studies induced alterations in the microenvironment. Changes in other components of the perivascular niche and relation to tumour cell location/number will be investigated using immunofluorescence protocols against endothelial markers CD31, CD34 and thrombospondin-1.

The data show that alteration of one component (vascular/bone) of the metastatic niche results in changes of the extended microenvironment. Our results highlight an association between components of the perivascular niche and the homing of breast cancer cells in the early steps of the metastatic process.

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P10.37

Clinical significance of EGFR-MET crosstalk for triple negative breast cancer

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Triple-negative breast cancers (TNBC) lack the three commonly used biomarkers such as oestrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor-2 (HER2). Partly due to the absence of treatment targets, TNBC remains one of the most aggressive forms of breast cancer. EGFR is overexpressed in 50–60% of the TNBC cases, unfortunately EGFR inhibitors are still ineffective. The Met receptor seems to play a role in the resistance towards EGFR inhibitors. We have quantified EGFR and MET gene copy numbers by droplet digital PCR in patient material and correlated our findings to available clinical data. TNBC often presented MET and EGFR amplification. These receptors, situated on chromosome 7, can be co-amplified in some tumours allowing us to hypothesise that a crosstalk between EGFR and Met could take place. A possible EGFR-Met interaction was investigated in vitro. Our preliminary results in TNBC cells showed that EGF (EGFR ligand) could activate the Met receptor. Further, we found that EGFR knockdown caused TNBC cells to proliferate more upon EGF treatment, mirroring the resistance to EGFR inhibitors in the clinic. However, dual transfection with EGFR and Met siRNAs inhibited cell proliferation and migration. These findings further support a crosstalk between these receptors and suggest that dual EGFR-Met inhibition could be a therapeutic alternative for TNBC. Another interesting finding was that EGF, when added at higher doses, inhibited cell proliferation and migration, contrary to what is expected from a growth factor. Changes in the EGFR signalling or expression can take place during metastatic progression. This has been mentioned in the literature as the "EGFR paradox" and the underlying mechanism is still unknown. In the clinical practice, it could be translated into a new possibility of treating certain EGFR positive tumours with EGFR agonists rather than antagonists.

P10.38

Role of Hes1 expression dynamics in cellular heterogeneity, plasticity and quiescence in breast cancer

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Non-genetic heterogeneity, cellular plasticity and quiescence are key clinical challenges in majority of the cancers, including breast cancer. A mechanistic understanding of these processes can lead to key therapeutic advances in the field of breast cancer.

Aiming to understand these processes at molecular and cellular levels, we have focused our attention towards understanding the role of expression dynamics of Hes1, a key target and readout of the Notch-Delta pathway. Up-regulation of Hes1 has been shown to be associated with metastasis, stemness, resistance to drugs and radiation and tumour cell quiescence in various cancers.

Hes1 shows a dynamic, oscillatory expression pattern during neural progenitor maintenance while its constant low expression leads to neuronal differentiation and its constant high expression possibly pushes these cells towards quiescence. Keeping in mind the role of Hes1 expression dynamics in neural stem cell fate decisions and also its deregulation in various cancers including breast cancer, we started exploring its role in breast cancer stem cell fate decisions, plasticity and quiescence. Our single cell analyses using fluorescent protein reporters tagging Hes1 show that its expression is oscillatory and dynamic in breast cancer cells; very importantly, kinetic parameters of these oscillations might function as a biological, cell-cycle length timer in these cells. On the other hand, analysis of Hes1 expression at the transcription level using small molecule RNA fluorescent in situ hybridisation (RNA smFISH) also showed that expression dynamics of Hes1 is variable between different classes of breast cancer stem cells, and also between cancer stem cells and non-stem cells.

Our work for the first time shows that Hes1 has a dynamic, oscillatory expression pattern in breast cancer model systems, and its expression dynamics are different between various cells constituting the tumour bulk (cancer stem cells and non-stem cells). We believe that the work has serious implications in understanding the key challenges in breast tumour biology such as cellular heterogeneity, plasticity and quiescence

P10.39

Rac1b regulates the quiescence and chemotherapy resistance of breast cancer stem cells in luminal breast cancer

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Rac1b is the constitutively active splice variant of the small GTPase Rac1, and is in part responsible for the hyperactivation of Rac1 signalling observed during breast tumourigenesis. Its expression is restricted to the embryonic development of various epithelial organs and ceases in adult life. However, upregulation of Rac1b expression has been observed in various cancers, including breast cancer. Here, we show that Rac1b is expressed only in luminal and Her2+, but not in triple-negative breast tumours, and its expression levels correlate with oestrogen receptor (ER) expression. We have analysed the loss-of- and gain-of-function phenotypes of Rac1b in the human breast cancer cell line, MCF-7, and demonstrated that Rac1b regulates the plasticity of the breast cancer stem cells and the response to chemotherapy. Rac1b-null transgenic MCF-7 cells, generated by CRISPR/Double-nickase approach, lost their tumour-formation capacity in vivo when transplanted into immune-deficient mice. Our results demonstrate that Rac1b may serve as a promising molecular target for developing novel breast cancer stem cell-targeting therapies for luminal breast cancer.

P10.40

Increased inducible nitric oxide synthase (iNOS) leads to increased risk of distant metastasis and poor survival in triple negative breast cancer

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Inflammation is implicated in triple-negative breast cancer (TNBC) progression. TNBC carries a worse prognosis than other breast cancer subtypes, and with the clinical and molecular heterogeneity of TNBC, there is a lack of effective therapeutic targets available. We have previously shown that inducible nitric oxide synthase (iNOS) is associated with increased p53 mutation, basal-like gene signature enrichment and transactivation of the epidermal growth factor receptor (EGFR) via s-nitrosylation, leading to poor outcome in oestrogen receptor-negative breast cancer. In this study, we investigated the role of iNOS on patient outcomes in 209 TNBC cases from the West of Ireland diagnosed between 2001 and 2015. iNOS tumour epithelial expression was associated with increased disease recurrence (Table 1), distant metastasis and decreased breast cancer-specific survival. Further investigation of potential tumour promoting mechanisms in triple-negative cell lines representing normal basal breast (MCF-10A), and basal-like 1 (MDA-MB-468) and basal-like 2 (HCC1806) tumours, showed that nitric oxide (NO) induces EGFR-dependent ERK phosphorylation in basal-like TNBC cell lines. Furthermore, NO-mediated cell migration and cell invasion were found to be dependent on EGFR and ERK activation particularly in basal-like 2 TBNC cells. This occurred in conjunction with COX-2 and NF-κB activation and increased secretion of pro-inflammatory cytokines IL-8, IL-1β and TNF-α. Treatment of TNBC xenograft mouse models with a combination of COX-2 inhibitors (aspirin or indomethacin) in combination with an iNOS inhibitor (aminoguanidine) led to decreased tumour growth suggesting a therapeutic role for combined iNOS– and COX-2-targeted therapy for the treatment of TNBC. Current research focuses on imaging strategies to create a 3D Atlas of the impact of tumour iNOS and NO on the tumour microenvironment milieu.

Multivariable Cox regression analysis

	HR	95% Confidence interval	*p* value	
iNOS levels and disease-free survival				
Negative	1.0			27
Low	3.5	1.04–11.51	0.044	79
Moderate	4.6	1.35–15.79	0.015	59
High	2.8	0.75–10.12	0.127	33

Adjusted for age at diagnosis, no chemotherapy/neoadjuvant/adjuvant chemotherapy/unknown, tumour grade, histological subtype

P10.41

Can IL-1 signalling be targeted to treat Notch-driven breast cancer?

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Breast cancer treatment options are currently limited for triple-negative breast cancer and therapy-resistant tumours. There is therefore a requirement to develop novel therapies targeting alternative cellular pathways, which could be used in combination with pre-existing treatments. The Notch signalling pathway has been found to be aberrantly activated in breast cancer, and is correlated with poor prognosis and therapy resistance. However, pan Notch inhibition is associated with significant side effects. Our group has found that Notch signalling induces the expression of IL-1α in breast cancer epithelial cells, resulting in the autocrine activation of pro-survival

Akt signalling. IL-1 signalling inhibitors are well tolerated and have an extensive safety record in the clinic. I therefore aim to determine whether IL-1 signalling can be targeted to treat Notch-driven breast cancers. Here, I present data which demonstrate the importance of IL-1 signalling in the breast cancer cell phenotype. IL-1R1 knockout MDA-MB-231 cells demonstrate reduced invasive capability, increased sensitivity to apoptosis and an inability to form tumours in the mouse xenograft model. Restoration of IL-1 signalling in the knockout cells restores the wild-type phenotype. Collectively the data show that IL-1 signalling plays an important role in the breast cancer cell phenotype and supports the hypothesis that IL-1 signalling could be therapeutically targeted in the treatment of breast cancer.

P10.42

Down-regulation of aquaporin 3 inhibits cellular proliferation, migration, and invasion in the MDA-MB-231 breast cancer cell line

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Aquaporins are membrane proteins that regulate cellular water flow. Recently, aquaporins have been proposed to be mediators of cancer cell biology. A subset of aquaporins, referred to as aquaglyceroporins, are also known to also facilitate the transport of glycerol.

This study describes the effect of gene knockdown of the aquaglyceroporin AQP3 on MDA-MB-231 breast cancer cell proliferation, migration, invasion, adherence and response to the chemotherapeutic agent 5-fluorouracil. shRNA-mediated AQP3 gene knockdown induced a 28% reduction in cellular proliferation ($p < 0.01$), a 39% decrease in migration ($p < 0.0001$), a 24% reduction in invasion ($p < 0.05$) and a 25% increase in cell death at 100 μM 5-FU ($p < 0.01$). Analysis of cell permeability to water and glycerol showed that MDA-MB-231 cells with knocked down AQP3 demonstrated a modest decrease in water permeability (17%, $p < 0.05$) but a more marked decrease in glycerol permeability (77%, $p < 0.001$).

These data suggest that AQP3 has a role in multiple aspects of breast cancer cell pathophysiology and therefore represents a novel target for therapeutic intervention.

P10.43

Breast Cancer Now Cell Culture Programme: a bespoke resource for the research community

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The Breast Cancer Now Tissue Bank (BCNTB) Cell Culture Programme aims to make a broad range of human primary breast cell populations and tissues available to the research community to more accurately model normal and malignant breast for a wide range of functional studies.

Most frequently, breast developmental and cancer research focuses on animal models or on long-established breast cell lines. Whilst these offer ease of use and reproducibility, they fail to represent the complexity of the human breast and its cellular and micro-environmental interactions, or its heterogeneity. BCNTB routinely consents and curates tissues from women undergoing surgery for cosmetic, risk-reduction or therapeutic purposes. Tissue is harvested fresh and processed for different experimental systems. Normal, risk-reduction (BRCA1/BRCA2/strong FH non-BRCA) and malignant tissues are enzymatically digested to single cells and populations purified using antibody-labelling techniques. Cells generated include normal epithelial, myoepithelial and fibroblast populations, as well as tumour-cell-enriched populations. Fibroblast populations include normal fibroblasts, tumour-associated fibroblasts and those isolated from matched morphologically normal adjacent (< 2 cm from tumour) and surround (> 5 cm from tumour) tissue. Unsorted single cells or undigested organoids are also banked. Culture conditions, including 3D co-culture, have been optimized for many of the cells. In addition to cells, live explants also are procured, representing both non-malignant and malignant breast. These have the advantage of maintaining physiological cell-stromal interactions, and can be cultured up to 7 days for tumour and 28 days for non-tumour tissue, providing systems to evaluate therapeutic and biological pathways. The Bank also provides intact fresh tissue for bespoke cell sorting or PDX. All samples have linked comprehensive clinico-pathological information and can be matched to tissue samples from the Tissue Bank. Collection of specific sample groups is available following discussion.

The ethos of the BCNTB Cell Culture Programme is to make available human materials that more accurately reflect the normal and diseased breast, in order to make research more physiologically relevant, and in doing so, more rapidly translate basic research into clinical relevance.

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Potential predictor genes for clinical outcome in triple negative breast cancer by next generation sequencing

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Background: The lack of therapeutic targets in the aggressive triple-negative breast cancer (TNBC) leads to unselective treatment of TNBC patients with cytotoxic chemotherapy. However, approximately 40% of systemic therapy naïve TNBC do not metastasize. This study aims at characterising TNBC transcriptome profiles by Next Generation Sequencing (NGS) for novel genes associated with distant metastasis (DMFS) and breast cancer-specific survival (BCSS).

Method: RNA-Seq analysis of a well-characterised series of TNBC was carried out using the HiSeq2500 instrument (Illumina, Inc.) using PE75 run chemistry. The targeted read count was 60 M total reads per sample. Row fast Q sequence reads files were quality and adapter processed using the trim galore wrapper for fast Qc and cut adapt, the resultant Qc reads were aligned to the hg38 (GRCh38.P5) build for the human genome using the Atlas aligner. Supervised artificial neuronal network (ANN) analysis of gene expression data was analysed to identify differentially expressed transcripts with respect to distant metastasis-free interval (DMFI) and breast cancer-specific survival (BCSS). The panel was further investigated by multivariate cox regression analysis to validate the transcripts.

Results: A comparative analysis looking for commonalities in the top 200 ranked gene predicting DMFI and BCSS (based on minimum average route mean squared error), identified 21 transcripts that show commonality in predicting poor outcome not only in terms of shorter DMFI but also shorter BCSS. Out of the 21 transcripts, Cox Regression revealed six genes were associated with poor prognosis ($p < 0.05$) independent of tumour size, grade, and nodal stage. Validation in external datasets is ongoing.

Conclusions: Our study identifies a six-gene panel of novel biomarkers that can independently predict prognosis and outcome in TNBC and can be a potential guide for management decision.