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Summary

The Annual IMPAKT (Improving Care and Knowledge through Translational Research) Breast Cancer Conference, held 07 to 09 May 2015 at The Square in Brussels, Belgium brought together leading investigators and experts in the field of breast cancer. The conference was organised by the European Society for Medical Oncology (ESMO) and the Breast International Group (BIG), in partnership with St. Gallen Oncology Conference and the European Breast Cancer Council.

Once again this meeting shined a spotlight on the newest basic and translational research that was applicable to all areas of breast cancer and held promise for new methods of diagnosis and treatment. To further this goal, IMPAKT 2015 offered a day of pre-conference training course covering topics that included the Basics of Cancer Biology, Tips and Tricks for Biomarker Research, Rapidly Evolving Research Areas, and Resources for Translational Research, all organised and taught by experts.

Symposia held throughout the conference provided cutting edge information on the molecular origin of breast cancer and the clinical significance of genomic patterns, the impact of mutations on therapy, the microenvironment and metastatic cascade in breast cancer, translational studies in early stage breast cancer, the role of chemotherapy in the context of precision medicine, and tackling the diversity of triple negative breast cancer.

Industry Satellite Sessions rounded out this learning experience and novel research findings were presented in posters and in oral presentations, which also provided state-of the-art information.

The scope of this report is to present the scientific highlights of the IMPAKT 2015 Conference.

Introduction

This year, 178 novel research abstracts were submitted. Importantly, the majority (48%) of abstracts focused upon prognostic, predictive and pharmacodynamic biomarkers in breast cancer that sought to provide aid in guiding clinical therapeutic decisions for this complex and pervasive disease. In addition, 19% of abstracts involved genomics and proteomic analysis of breast cancer, important tools for elucidating the mechanism of breast cancer and metastasis, and for identifying biomarkers and potential drugable targets. Abstracts on loco-regional therapy, advanced breast cancer systemic therapy and preclinical breast cancer biology each accounted for 15% of accepted abstracts, whereas 10% of abstracts each focused on detection and diagnosis of breast cancer. Other categories of abstracts included breast cancer host immune and stromal biology, breast cancer target identification, validation and preclinical models and early breast cancer systemic therapy, with each topic accounting for 7% of all abstracts. Five percent of presented abstracts centred on imaging and an equal 5% targeted new drug development. The remaining abstracts were categorised as miscellaneous but every abstract in each category created a pool of new data and a dialogue that provided new insight into clinical practice and renewed inspiration for investigators.

Over 553 participants came from 55 countries worldwide to share and learn about the most up to date technological advances, research findings and clinical care strategies in the field of breast cancer. This year saw a 3.6% overall rise in attendance over the previous conference, with 464 delegates being joined by 41 invited speakers, 20 Industry Exhibitors and 8 members of the Press. Travel grants were awarded to 20 participants.

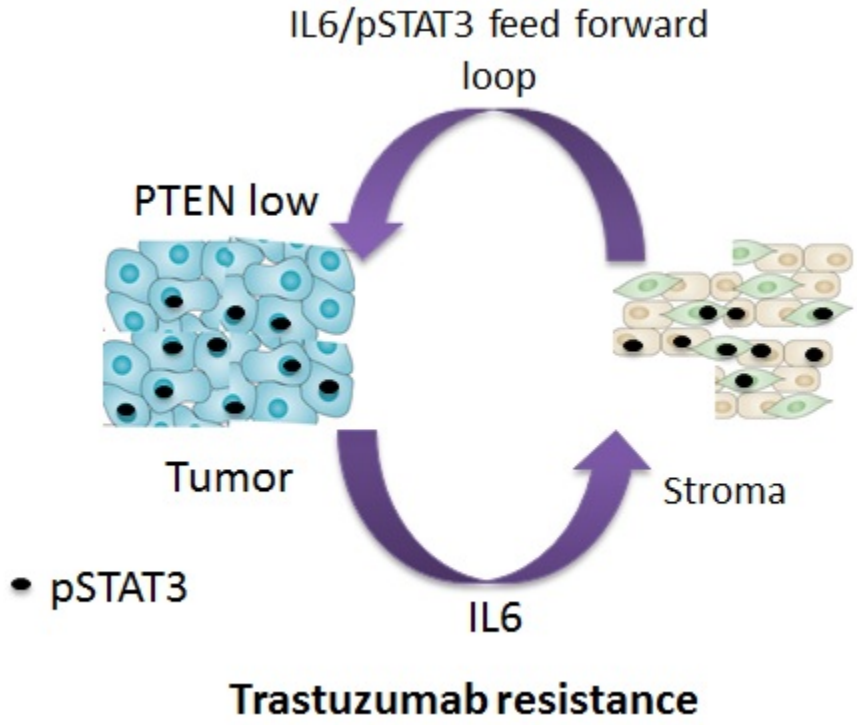
The 10 most represented countries were Belgium, USA, UK, Italy, Iran, France, Germany, Spain, Switzerland, and Austria, which comprised 68% of attendance. In all, 55 countries were represented with 32% of the participants coming from other European counties, North and South America, Asia, The Middle East, Africa, and Australasia. The average age of attendees was 43 years but individuals of all ages, from early 20’s to over 65 years participated in this years’ conference.

Nearly a quarter of attendees (23.1%) were medical oncologists, followed by basic researchers/scientists (15.1%), surgical oncologists (11.3%), and trainees/residents in medical oncology (7.5%). Other participants of the more than 30 professions represented were pathologists, clinical oncologists, medical staff, PhD students, biologists, gynaecologists, nuclear medicine practitioners, and clinical geneticists, just to name a few of the diverse professionals that contributed to the intellectual and professional character of the audience.

This conference provided an opportunity for participants to meet top experts in the breast cancer field, share information, and form new collaborations. Attendees left with the updated knowledge necessary to provide the best medical care available to their patients with breast cancer.

Constitutively activated STAT3 may signal trastuzumab resistance in patients with primary HER2 positive breast cancer

Trastuzumab is an effective treatment for disease recurrence often experienced by patients with HER2 positive breast cancer following successful first-line treatment. However, many patients have tumours that show resistance to trastuzumab, according to Amir Sonnenblick, Breast Cancer Translational Research Laboratory (BCTL), Institute Jules Bordet, Brussels, Belgium, leading his team to evaluate a mechanism for trastuzumab resistance and to identify molecules expressed on tumours that may confer resistance to trastuzumab. The investigators hypothesised that expression of phosphorylated Signal Transducer and Activator of Transcription 3 (P-STAT3), which is constitutively activated in approximately 30% to 40% of breast cancer, may associate with trastuzumab resistance and, therefore, may be predictive of poorer response to this breast cancer therapy.



Caption: There is a potential link between IL6-pSTAT3-PTEN loss, stromal reactivation and primary trastuzumab resistance in HER2-positive primary breast cancers. © Amir Sonnenblick

The investigators identified a P-STAT3 associated gene signature (P-STAT3-GS) in an independent dataset (TCGA) that predicts P-STAT3 status; this characteristic set of P-STAT3 dependent induced changes may play a role in HER2 positive cancers, area under the curve (AUC) = 0.78 (p = 0.01). Tumours with high levels of P-STAT3-GS were found to associate with

trastuzumab resistance (log rank p = 0.49). Upon statistical analysis, samples of oestrogen receptor negative tumours showed a strong relationship between P-STAT3-GS and trastuzumab resistance (interaction test p = 0.02).

They then used data from the fin-HER prospective randomised controlled study to confirm this association. In this study, data from protein evaluated by reverse phase protein array were integrated with gene expression data from 95 HER2 positive breast cancer samples from patients treated with trastuzumab in the adjuvant setting. This “Responsify” dataset showed decreased time to distant metastasis in patients with tumours expressing high levels of P-STAT3 compared to those with low expression in the overall cohort (p = 4.9e-02). Patients expressing high versus low P-STAT3 and were oestrogen receptor negative (ER-negative) showed a comparatively shorter time to metastasis (p = 3.9e-03), whereas patients with ER-positive tumours responded with similar time to metastasis regardless of the level of pSTAT3 expression (p = 7.6e-01). The P-STAT3-GS was found to associate with clinical outcome; in the overall population more patients with low versus high P-STAT3-GS expression achieved distant metastasis-free survival at 6 years with trastuzumab versus no trastuzumab (p = 1.3e-01) and this result was more pronounced in patients with ER-negative tumours (p = 9.9e-03). However, patients with high P-STAT3-GS expression showed similar rates of distant metastasis-free survival at 6 years with and without trastuzumab (p = 4.8e-01 and p = 9.7e-01, respectively), indicating patients had developed resistance to trastuzumab.

When subjected to false discovery rate (fdr) analysis, which allows for multiple comparisons, constitutively activated P-STAT3 in tumours associated with loss of PTEN (r =- 0.4, fdr = 0.025), elevated interleukin 6 (IL6) (r=0.4, P = 4.72e−05) and stromal reactivation. The authors suggest that targeting the STAT3 pathway may be the way forward to restore sensitivity to trastuzumab in patients with HER2 positive breast cancer demonstrating trastuzumab resistance.

Dr Lisa Carey of the University of North Carolina, USA discussed the study results, noting that STAT expression segregates HER2-positive breast cancers into two molecularly distinct groups: luminal and HER2-enriched, with HER2-enriched having relatively low STAT3 expression. She commented that P-STAT3 status is relevant for HER2-targeting only in ER-negative disease, and ER-negative tumours comprise more than 50% of HER2-enriched tumours. She remarked that pSTAT3 association with stromal reactivation is an intriguing finding, given that stromal reactivation and the epithelial-mesenchymal transition are both associated with drug resistance. Due to the explosion of good (but expensive) options for HER2-targeting, we must find out who needs more from the treatment and who needs less, according to Dr. Carey. Sonnenblick et al. Abstract 39O.

Practice point and future research opportunities

Expression of a phosphorylated Signal Transducer and Activator of Transcription 3 protein associated gene signature (P-STAT3-GS) associates with trastuzumab resistance in patients with breast cancer and may serve as a marker for patients that will no longer benefit from this treatment.

Gene expression signatures indicate palbociclib resistance and sensitivity in patients with breast cancer

Findings that may guide clinicians in selecting which patients will show a response to palbociclib were presented by Ilenia Migliaccio Translational Research Unit, Hospital of Prato, Istituto Toscano Tumori, Prato, Italy, who headed an Italian team in detecting markers of response to palbociclib, which is very promising for the treatment of patients with oestrogen receptor positive, human epidermal growth factor receptor 2 negative (ER-positive HER2-negative) tumours. Palbociclib works by inhibiting protein kinase 4 and 6 (CDK4/6) that play roles in cell division and proliferation. However, resistance to palbociclib has begun to appear and is known to be heralded by retinoblastoma (RB) genetic loss, leading the investigators study whether a genetic signature of functional RB loss would predict resistance to palbociclib and also to identify other gene expression signatures that would be predictive of sensitivity to palbociclib.

These signatures were tested for prognostic and predictive value in a broad breast cancer gene expression meta-dataset (3458), and the ability of these signatures to discriminate between palbociclib sensitive or resistant cell lines was also tested using a validation dataset of breast cancer cell lines (GSE48213), according to a previous procedure by Finn et al. (PMID:19874578).

Several tests revealed that the 87 gene RB signature was predictive of RB status in the TCGA dataset across all molecular subtypes. Previously untreated patients with breast cancer and ER-positive tumours that also expressed high levels of the RB signature denoting loss of the gene demonstrated significantly worse recurrence free survival (RFS) compared to similar patients with tumours expressing low levels of the RB signature; hazard ratio (HR) 2.34 ($p = 7.5e{-}09$) and endocrine only treated patients with similar expression levels, HR 2.58 ($p = 5.4\ e{-}11$), respectively. In the validation dataset, the RB signature was a strong predictor in evaluating sensitivity or resistance to palbociclib; ROC area under curve (AUC) = 0.93. The second gene signature, PDSENSsig was composed of 20 genes found to be upregulated in sensitive cell lines and was identified by comparing 13 palbociclib sensitive and 13 palbociclib resistant cell lines in the Cancer Cell Line Encyclopaedia (CCLE) dataset. Untreated or endocrine treated patients with ER-positive tumours expressing high PDSENSsig had significantly better RFS compared to those with low PDSENSsig, HR = 1.42 (95% CI 0.47, 1.93; (logrank p = 0.0038 and HR = 1.71; 95% CI 1.26, 2.50 (logrank p = 1e-04), respectively. A good performance of the PDSENSsig predictor was obtained in the validation dataset (ROC AUC = 0.76). The third gene signature, PDRESsig was predictive for resistance; untreated or endocrine treated patients with ER-positive tumours expressing high PDRESsig had significantly poorer RFS compared to those with low PDRESsig, HR = 1.42 (95% CI 1.04, 1.90; (logrank p = 0.028 and HR = 1.71; 95% CI 1.26, 2.37 (logrank p = 5e-04), respectively.

In discussing the study, Dr Lisa Carey of the University of North Carolina, USA, pointed out that clinical trials of palbociclib in breast cancer patients, such as the PALOMA1 trial, have suggested that screening for CCND1 amplification or p16 loss does not seem to improve patient selection, resulting in a low clinical benefit rate of palbociclib monotherapy in a ER-positive, RB-positive heavily pre-treated population, underscoring the need for additional studies to better

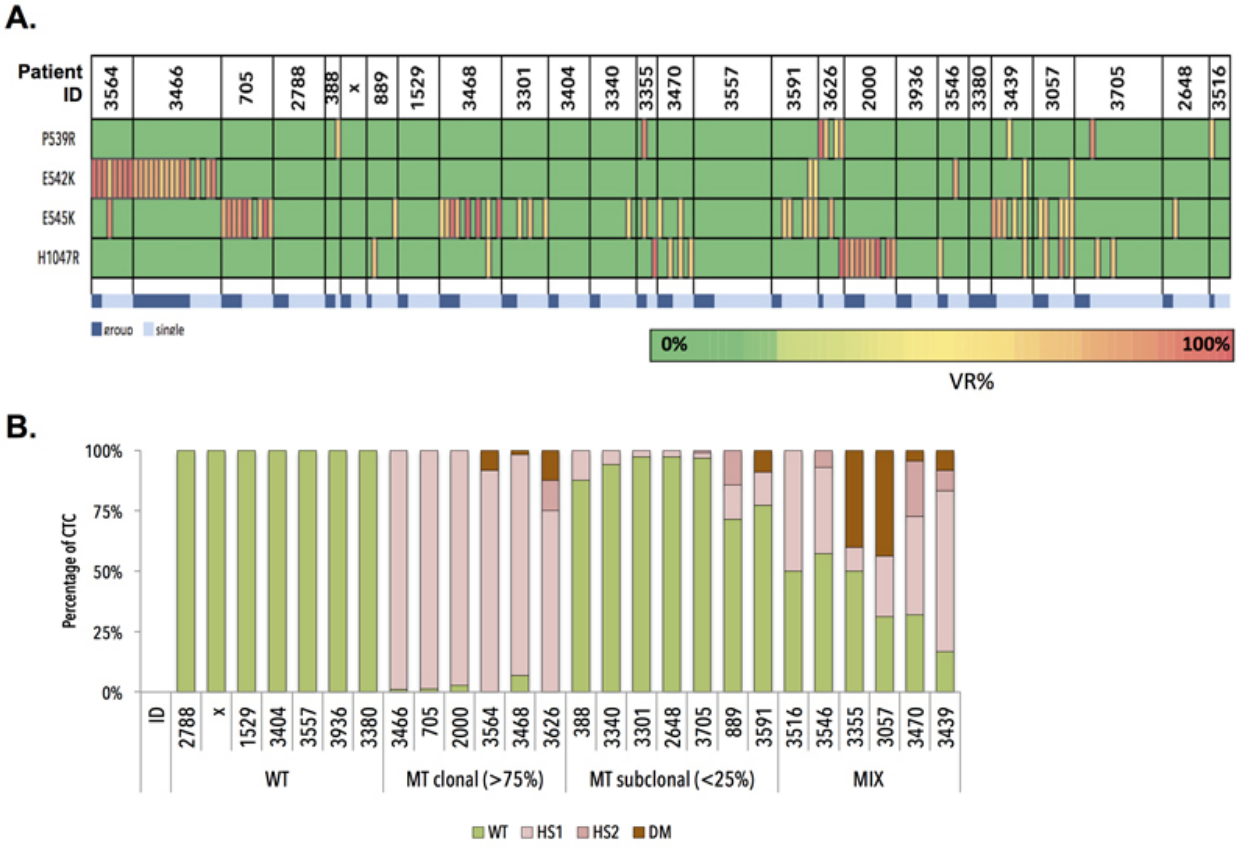
identify palbociclib sensitive/resistant patients. She noted highlights of the study, which included that the functional RB loss signature correlates with prognosis for luminal B tumours, which she called plausible and consistent, but noted that CDK4/6 inhibitor resistance may relate to subtype and proposed that the signature based on E2F may reflect proliferation more than RB. She raised the issue that, while not validated, it was an important finding of this study that palbociclib sensitivity and resistance signatures can be created and modestly track with expected phenotype and behaviour. She questioned, however, whether these signatures will be better than just the RB-based assay and pointed out that predictive biomarkers are important for this emerging class of drugs and known variable such as subtype, may be needed to be taken into account as well. Migliaccio et al. Abstract 40O

Practice point and future research opportunities

This study identified biomarkers that may be helpful in selecting patients more likely to benefit from palbociclib treatment. Three gene expression signatures were identified and tested in this study that showed good predictive value of response and/or sensitivity to palbociclib. The expression of these signatures in tumours from breast cancer patients associated with response to treatment with palbociclib. Further validation in cohorts of patients with breast cancer treated with palbociclib is warranted, especially to determine if they offer superior predictive value over the RB-based assay.

Non-invasive mutational analysis of PIK3CA status in circulating tumour cells developed for patients with metastatic hormone receptor-positive breast cancer

Another step forward towards the non-invasive evaluation of response to treatment and disease progression in patients with metastatic hormone receptor-positive breast cancer was taken in a new study. Bram De Laere, Centre for Oncological Research - Campus GZA Sint Augustinus, University of Antwerp, Antwerp, Belgium reported that a tumour cell liquid biopsy was used successfully to evaluate the mutation frequency of the PIK3CA gene. The team conducted a large analysis of the PIK3CA genotype in circulating tumour cells (CTCs). CTCs could be detected in the blood of patients with metastatic hormone-receptor positive breast cancer and detection was sensitive to the single cell level. The investigators focused on PIK3CA status, as it is frequently mutated in hormone receptor-positive metastatic breast cancer and may change between early and advanced disease; therefore, it may be an important harbinger of disease progression. The frequency of PIK3CA mutations in CTCs were compared with primary tumour samples obtained from each patient to establish whether CTCs provided an accurate depiction of the PIK3CA mutational status of the primary tumour.



Caption: PIK3CA mutational analysis in CTCs of patients with metastatic hormone receptor-positive breast cancer reveals intra-patient homo- and heterogeneity. © Bram De Laere

CellSearch and DEPArray techniques were used to purify 249 single CTCs and 148 groups of CTCs (range: 5 to 120 cells per group) and 58 white blood cells (WBC; range: 4 - 20) from the peripheral blood of 29 patients with metastatic oestrogen receptor-positive/progesterone receptor-positive/HER2-negative breast cancer. The investigators also purified circulating cell-free DNA (cfDNA) from cell preparation tube (CPT)-collected plasma and genomic DNA from archival formalin-fixed paraffin embedded (FFPE) tissue sections from primary tumour as comparators. Mutation analysis was performed by targeted 454 pyrosequencing.

PIK3CA mutations were present in 59.2% of archival primary tumour samples and showed poor agreement with 21 cfDNA samples (42.8% disparity; κ=0.113) and fair agreement with 22 CTCs (27.2% disparity; κ=0,394). Comparison of 22 each of CTCs and temporally matched cfDNA samples revealed moderate agreement (22.7% disparity; κ=0.409). A concordant PIK3CA status across all compartments was observed in 55.5% of 18 samples. At the sequencing depth used in the study, cfDNA failed to detect PIK3CA mutations in 4 (22.2%) cases and plasma did not detect mutation in 2 of 18 (11%). Disparity in PIK3CA status between early and advanced disease was found in 4 (16%) patients. Gain of mutation was observed in 4 (22.2%) of 18 patients having a wild-type primary tumour plus mutant CTCs and cfDNA upon disease progression.

In depth analysis performed among the CTCs revealed PIK3CA mutations that demonstrated both homo- and heterogeneity with cases, which suggested the presence of both mutant and wild-type CTCs in the population. Additionally, unique double-mutated CTCs were detected in 10 (38.4%) patients.

Prof. W. Fraser Symmans from the University of Texas M.D. Anderson Cancer Centre, Houston, USA, who discussed the study results, remarked that feasibility was demonstrated for single CTC measurements and that PIK3CA genomic status in tumour tissue appears to be more concordant with status in CTCs than with status in cfDNA. He noted that the PIK3CA genomic status was sometimes different in the circulation from the status in the tumour tissue and this was observed more often in tumour tissue with wild-type status. It may be more common with cfDNA than with CTCs. De Laere et al. Abstract 41O

Practice point and future research opportunities

Evaluation of the PIK3CA status in a non-invasive real-time manner is important since this status may change between primary and metastatic disease. In this study, analysis of CTCs was more useful than plasma analysis to assess PIK3CA mutational status, with CTCs showing better agreement with the primary tumour sample DNA in terms of mutational status. A CTC liquid biopsy could pave the way towards applying an optimized personalised medicine in the management of patients with metastatic cancer; however more data from a larger study with matched primary and metastatic tissues, and blood samples are needed.

HORMAD1 identified by genomic profiles as a driver of homologous recombination deficiency and platinum therapy response in triple-negative breast cancer

Anita Grigoriadis, Breakthrough Breast Cancer Research Unit, King's College London Guy's Hospital, London, UK, and colleagues carried out the study to determine the possible aetiology and consequences of as yet undefined genomic patterns giving rise to the complex numerical and structural DNA alterations that characterise triple negative breast cancers (TNBC), other than those of the homologous recombination (HR) repair proteins BRCA1 and BRCA2. The investigators aimed to identify and functionally validate a candidate driver of specific genomic instability forms in TNBC: to do so they established 3 measures of chromosomal instability scarring (SCINS): allelic imbalanced copy number aberrations (AiCNAs); copy neutral losses of heterozygosity (CnLOH); and allelic balanced copy number aberrations based on SNP microarray allele-specific copy number profiles, and applied them to 331 TNBC samples, 38 breast cell lines, and 299 The Cancer Genome Atlas (TCGA) high-grade serous ovarian tumours.

The investigators identified 3 groups of TNBC samples with distinctive genomic patterns: those high in AiCNA, those high in CnLOH, and a third group that was low for all SCINS measures. High levels of HORMAD1, a cancer testis antigen involved in double strand DNA break processing during meiosis, were revealed by gene expression analysis in the groups having

high AiCNA and CnLOH. AiCNA was also shown to correlate with HORMAD1 protein expression, which was confirmed by Western blot and immunohistochemistry. Immunofluorescence validated that HOMAD1 is located in the nucleus.

When HORMAD1 was over-expressed in cell lines, cytogenetic abnormalities and AiCNA increased. DR-GFP and EJ5 non-homologous end-joining (NHEJ) reporter assays revealed reduced HR and increased NHEJ efficiency in these cells. Cells wherein HORMAD1 expression was induced were sensitised to cisplatin and to two PARP inhibitors.

In the PrECOG0105 trial, which was a neoadjuvant platinum chemotherapy trial, bimodality analysis identified HORMAD1-low and -high expressing tumours; tumours expressing high levels of HORMAD1 were more likely to respond to treatment in both unselected and BRCA1/2 wildtype TNBC ($p = 0.008$).

Discussant W. Fraser Symmans from the University of Texas M.D. Anderson Cancer Centre, Houston, USA, commented: “The translational implications of this early and innovative work are very interesting that has an exciting potential for wild-type BRCA 1/2 triple negative breast cancer,” adding that BRCA1/2 aberrations lead to genomic instability, as indicated by SCINS. He noted HORMAD1 activity could be possibly a response to DNA damaging treatment. Grigoriadis *et al*. Abstract 42O

Practice point and future research opportunities

The measures of chromosomal instability scarring done in this study were useful in defining HORMAD1 as a potential driver of specific patterns of genomic instability and as a possible biomarker for sensitivity to platinum and two PARP inhibitors due to the ability of the HORMAD1 protein to induce homologous recombination deficiency and upregulation of non-homologous end-joining.

Triple therapy may overcome resistance to palbociclib in ER-positive breast cancer

The combination of endocrine therapy, plus inhibitors of PI3 kinase and CDK4/6, is highly active and may be the way forward to treat patients with oestrogen receptor (ER) positive breast cancer and acquired resistance to palbociclib, according to results presented during the Best Abstracts session. Maria Teresa Herrera-Abreu, Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, UK and colleagues located in Europe and the USA demonstrated that response can be restored in palbociclib-resistant cell lines and elucidated the complex mechanism by which these agents work in concert to improve the efficacy of or restore sensitivity to palbociclib and other CDK4/6 inhibitors. The team used compound sensitivity screening to identify drugs that were sensitized with palbociclib, an inhibitor of cell division protein kinase 4 and 6 (CDK4/6), which mediates cell division and proliferation. They further investigated the mechanisms of this sensitization both in vitro and in vivo. The investigators reasoned that, in this way, an agent that could work synergistically with palbociclib providing increased clinical activity could be uncovered.

While cell proliferation in ER-positive breast cancer is driven by cyclin D1-CDK4/6 inhibition of this molecule alone fails to induce a durable cell cycle arrest and early adaptation to CDK4/6 inhibition by palbociclib or other agents is caused by low level cell cycle re-entry due to cyclin D1 accumulation induced by PI3K signalling. Cell cycle arrest can be prolonged by a combination of CDK4/6 and PI3K inhibition; a PI3 kinase/mTOR inhibiting agent, GDC0941, increased the sensitivity of ER-positive breast cancers to palbociclib inhibition of CDK4/6 that allowed a complete block to cell cycle re-entry driven by cyclinD1 and converted the cytostatic arrest of CDK4/6 inhibition into a profound cytotoxic effect. This combination was efficacious in vitro and in ER-positive breast cancer cell lines.

While early loss of cell cycle inhibition with palbociclib could be improved by dual targeting of PI3K plus CDK4/6 and showed even stronger inhibition when the ER was also targeted with fulvestrant, late acquired resistance was not salvaged by dual targeting. Acquired resistance to CDK4/6 inhibition is due to loss of cyclin D1 dependence, by either RB1 loss or cyclin E1 amplification. The investigators found that palbociclib resistant cell lines did not benefit from just the combination of palbociclib/GDC0941, underscoring the need for new therapeutic strategies for the treatment of palbociclib resistant cancers. A phase I trial of triple targeting of ER/PI3K/CDK4/6 is underway.

Dr Lisa Carey of the University of North Carolina, USA, who discussed the study results, said that it may be necessary to use rational combinations at an early stage and comprehensively, since cell cycle inhibition erodes and resistance to these drugs can be developed. The study finding that CDK2 is implicated in both early and late (acquired) resistance is consistent with other reports. She noted that the effects of co-targeting PI3K plus CDK4/6 are at least additive but works only against early adaptive resistance; this finding is also consistent with work done using a CDK4/6 inhibitor to circumvent resistance to PI3K inhibition. Herrera-Abreu *et al.* Abstract 86O

Practice point and future research opportunities

Findings from resistant cell lines used in this study demonstrated that the combination of PI3 kinase and CDK4/6 inhibitors is highly active in ER-positive breast cancer, and acts to convert the cytostatic arrest of CDK4/6 inhibition into a profound cytotoxic effect. The strategy of triple targeting of ER/PI3K/CDK4/6 works the best to overcome early resistance.

Breast cancer specialists vary on risk of recurrence evaluation and administration of adjuvant chemotherapy

Caroline Drukker, Surgical Oncology, The Netherlands Cancer Institute Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands, and colleagues surveyed breast cancer specialists to formulate the consensus among them regarding the degree of recurrence risk and whether or not to administer adjuvant chemotherapy to an individual patient, plus the extent to which they adhered to current clinical guidelines. The online based risk-cut off values used in the MINDACT study control arm were used for evaluation of results from the survey that also questioned which combinations of clinical / pathological features figured in the specialist's opinion. The intent was to use this information to fine-tune the analysis of MINDACT results.

Data from 40 participants in the MINDACT trial were evaluated by 82 specialists who estimated the risk of recurrence (high or low) for each case and provided a recommendation for adjuvant chemotherapy. The format was an online questionnaire and cases were randomly reordered between participants. Agreement both among the specialists and with the MINDACT control arm was evaluated; agreement among breast cancer specialists was measured per individual case as the frequency of the most frequent assessment for that case, ranging from 50% (complete disagreement) to 100% (perfect agreement) and the agreement with the control was evaluated as the amount of assessments that are the same as the MINDACT control, ranging from 0% (complete disagreement) to 100% (perfect agreement). Overall agreement equalled the weighted average of the case-agreements by both agreement types.

Each case was evaluated by a median of 73 breast cancer specialists (range: 71 to 76). Of the respondents, 78% were medical oncologists, with 76% having at least 10 years of experience. There was moderate (77%) overall agreement among participants on the risk of recurrence and the administration of adjuvant chemotherapy; however there was high variation from case to case (range: 53% to 100%). Less overall agreement between the participants and MINDACT control was seen (64%) and variation across cases was broad (range 5% to 100%). The investigators are continuing to map the areas of patient characteristics carrying most of the disagreement, and are evaluating whether gene signatures may affect the consensus. Drukker *et al.* Abstract 2P**.** NCT00433589

Practice point and future research opportunities

Breast cancer specialists showed low to moderate agreement regarding the risk of recurrence and administration of adjuvant chemotherapy on a per patient basis and also with the MINDACT control arm.

Enzalutamide shows benefit in a subset of patients with triple negative breast cancer and tumours expressing the androgen receptor

Javier Cortes, Vall d'Hebron University Hospital Institut d'Oncologia, Barcelona, Spain and colleagues tested enzalutamide in women with triple negative breast cancer (TNBC) tumours that expressed the androgen receptor (AR). Enzalutamide is a potent oral inhibitor of AR signalling that improves overall survival (OS) in men with metastatic castration-resistant prostate cancer. AR expression has been observed in 10 to 30% of patients with TNBC. In addition, evidence from cell lines of patients with TNBC expressing the AR that showed a response to enzalutamide inhibition of androgen stimulation led to this trial. MDV3100-11 was an open-label, Simon 2-stage study of enzalutamide in patients with TNBC and AR expression ≥10% by centrally reviewed immunohistochemistry plus a response assessment. The primary endpoint was the clinical benefit rate at 16 weeks (CBR16).

More than 400 tissue TNBC samples were evaluated; 79% of samples were positive for AR expression and AR expression was ≥10% in 55% of samples. Preliminary findings showed that 77% of the 26 patients with evaluable data had measurable disease, 62% had visceral involvement, 69% had ≥3 metastatic sites, and 35% of patients had received ≥3 prior treatment regimens.

The trial met its prespecified efficacy endpoint; CBR16 was 42% (95% confidence interval (CI) 24.2%, 61.9%) and CBR24 was 35% (95% CI 18.3%, 54.2%). One patient (7.7%) each demonstrated partial response, (PR) and complete response (CR). Median progression-free survival (PFS) was 11.5 (95% CI 7.4, 27.4) weeks. In the intent to treat population (ITT), median CBR16 in 42 patients was 28.8% (95% CI 15.95%, 43.9%) and median CBR24 was 23.8% (95% CI 12.3%, 39.0%). One (4.8%) patient each achieved CR and PR. Median PFS in the ITT population was 19.5 (95% CI 10.4, 50.9) weeks.

Adverse events (AEs) occurring in ≥15% of all patients in the ITT population were fatigue, which was seen in 15 (36%) patients, nausea in 14 (33%), diarrhoea in 9 (21%) and decreased appetite, which was reported by 8 (19%) patients. AEs ≥ grade 3 were fatigue, which was reported by 3 (7.1%) patients, anaemia in 2 (4.8%), and nausea and vomiting, which were reported by one (2.4%) patient each. Treatment emergent adverse events (TEAs) were reported by 26 (66.7%) patients overall receiving enzalutamide. TEAs leading to study discontinuation occurred in 3 (7.1%) patients and 2 (4.8%) patients had a TEA resulting in death. Cortes *et al.* Abstract 13P.

The largest prospective clinical trial conducted to date of enzalutamide in patients with androgen receptor (AR) positive TNBC demonstrated benefit from anti-AR therapy in these patients who showed an objective response.

Promising phase Ib study results seen with pembrolizumab in patients with metastatic triple negative breast cancer

Lead investigator Laurence Buisseret, Institute Jules Bordet, Brussels, Belgium presented results from a cohort of patients with metastatic triple negative breast cancer (mTNBC) participating in the KEYNOTE-012 trial of the efficacy and safety of pembrolizumab in patients with mTNBC, advanced gastric cancer, urothelial cancer, and head and neck cancer.

Since pembrolizumab is a humanized IgG4 monoclonal antibody that targets programmed death receptor 1 (PD-1), the investigators first screened 111 patients with evaluable mTNBC samples for PD-L1 expression; PD-L1 positive samples were defined as staining in the stroma or in ≥1% of tumour cells as assessed by a prototype immunohistochemistry assay using the 22C3 antibody. Screening results showed that 65 (59%) patients had PD-L1–positive tumours. In all, 32 patients with a mean age of 52 (range: 29 to 72) years were enrolled, 78% of patients were White and 22% of patients were Black. All patients had received prior treatment, with 46.9% of patients receiving ≥3 prior lines of therapy for metastatic disease; 100% of patients had received taxanes.

By November 10, 2014, 27 patients were evaluable for response per RECIST v1.1 by central review within a median follow-up of 10.4 (range: 0.4 to 16.1) months. The median time to response was 18 (range, 7 to 32) weeks after receiving pembrolizumab at 10 mg/kg every 2 weeks. Women with heavily pre-treated mTNBC showed clinical benefit following pembrolizumab, including a complete response that was durable; the objective response rate (ORR) was 18.5% (95% confidence interval (CI) 6.3%, 38.1%). Complete response (CR) was observed in one (3.7%) patient, 4 (14.8%) patients achieved partial response (PR), and 7 (25.9%) patients experienced stable disease for a confirmed ORR of 18.5%. Progressive disease occurred in 12 (44.4%) patients and 3 (11.1%) were not available to evaluate. The progression-free survival (PFS) rate at 6-months was 25.9% and the median PFS was 1.9 (95% CI 1.7, 5.5) months.

A reduction in target lesion from baseline was also demonstrated by 36.4% of patients. The median duration of response was not reached (range, 15 to 40+ weeks) and 3 responding patients have remained on treatment for ≥11 months. At baseline, 27 patients had known lactate dehydrogenase levels (LDH); disease progression occurred within 60 days in all 5 (100%) patients with LDH levels of 800 IU/L or greater and in 11 of 22 (50%) patients with LDH levels less than 800 IU/L.

Treatment-related AEs (TRAEs) occurred in 56.3% of patients; the most commonly reported were fatigue, myalgia, and nausea, which were each reported by 18.8% of patients. TRAEs

grade 3 to 5 of anaemia, aseptic meningitis, blood fibrinogen disease, disseminated intravascular coagulation, headache, and pyrexia each occurred in one (3.1%) patient. One case of treatment-related disseminated intravascular coagulation leading to death occurred on study. No patients discontinued study due to AEs. Buisseret *et al.* Abstract 14P. EudraCT number 2012-005771-14.

Practice point and future research opportunities

Pembrolizumab is already approved for the treatment of melanoma, one of the many tumour types that co-opt the programmed death receptor 1 pathway to evade surveillance. The ongoing development of pembrolizumab for the treatment of metastatic triple negative disease in patients with PD-L1 positive tumours is supported by these findings wherein patients, even those receiving 5 or more prior lines of treatment for advanced disease, showed responses and achieved stable disease.

Third-line eribulin lowers levels of circulating tumour cells in patients with metastatic breast cancer

Luis Manso, Clinical Oncology, University Hospital 12 De Octubre, Madrid, Spain presented data from the ONSITE trial that monitored disease status using circulating tumour cell (CTC) levels following eribulin infusion at 1.23 mg/m2 on days 1 and 8 of every 21-day cycle as third-line therapy in patients with human epidermal growth factor receptor 2 (HER2)-negative recurrent or metastatic breast cancer. CTCs were measured per 7.5 mL of blood obtained at baseline and after the first cycle of treatment using the CellSearch System, Veridex LCC. The study comprised 53 patients with breast cancer that had progressed following treatment. The patients were 98.1% female with a mean (standard deviation) age of 56.9 (12.9) years. Taxanes had been administered previously to 88.7% of patients and 73.6% of patients had received anthracyclines. Receptor status was 64.2% oestrogen receptor positive, 58.8% progesterone positive, and 15.4% were triple negative.

The mean (standard deviation) value of baseline CTCs in 44 patients was 17.4 (27.7) cells compared to 5.9 (11.2) cells detected following two cycles of eribulin ($p = 0.002$). A baseline value of CTCs > 5 was seen in 20 (37.7%) patients that decreased to 13 (24.5%) patients after treatment ($p = 0.035$). The number of patients with CTC levels <5 increased from 45.3% at baseline to 50.9% after treatment, with 63% of patients having CTC values < 5 after two cycles of eribulin.

At a follow-up done after 3 months of treatment in 26 patients, the best clinical response by RECIST criteria showed 9% of patients achieved partial response (PR), 21% of patients had stable disease (SD), and 18% of patients had progressive disease. No patients achieved complete response. Ten (55%) patients showing a response of PR or SD maintained a CTC value < 5 post-treatment. Manso *et al.* Abstract 19P. Clinical trial identification: EudraCT number 2012-005771-14

The results suggest that third line treatment with eribulin was related to a significant reduction in levels of CTCs, indicating an improvement in prognosis. Patients also showed clinical benefit. These findings support the use of third-line erubulin in patients with metastatic breast cancer.

Specific subset of patients with HER2 negative breast cancers and HER2 positive circulating tumour cells benefit from HER2 positive targeted therapy

Christian Kurbacher, Gynaecologic Centre Bonn-Friedensplatz, Bonn, Germany and colleagues conducted a study of anti-HER2 therapy in women with metastatic breast cancer determined to be human epidermal growth factor receptor 2 negative (HER2-negative) but who have high levels of HER2-positive circulating tumour cells (CTCs) or proteins in their serum.

According to Professor Kurbacher, these “occult” HER2-positive patients are not usually offered anti-HER2 therapy but it was unknown whether they may benefit from this treatment. He noted that large-scale clinical trials, such as DETECT-II, have been set up to address this issue but results have not yet been reported, so his team initiated this observational study to gain more insight into the feasibility of HER2-positive directed therapy in this cohort.

This study comprised 25 patients with HER2-negative metastatic breast cancer; 21 patients were oestrogen receptor positive. All patients had been heavily pre-treated and had received a median of 7 (range: 2 to 16) prior systemic therapies. The patients had a median age of 52.5 (range: 35 to 84) years. Six patients were serum HER2-positive with values greater than 15 ng/mL, as determined by a commercial chemiluminescence immunoassay, 6 patients had HER2-positive CTCs, and 13 patients were both serum positive and had HER2-positive CTCs. Anti-HER2 treatment included trastuzumab in 13 patients, lapatinib in 4 patients, trastuzumab plus lapatinib in 2, and trastuzumab plus pertuzumab in 6 patients. HER2 directed therapy was given for a median 14.6 weeks.

The objective response rate (ORR) according to RECIST 1.1 was 40%; 10 patients achieved partial response (PR) and 10 patients experienced stable disease (SD). Progressive disease (PD) occurred in 20% of patients and there were no complete responses. The rate with benefit (RWB) was 80%. Median overall survival was 62.9 weeks.

After 3 weeks of treatment, serum HER2-positive levels decreased from baseline by more than 20% in most patients; all patients with PR and SD showed decreased CTC values and most had normal values within 6 weeks. All patients with PD showed increasing CTC values. Two patients that were treated with lapatinib and achieved PR showed an increase in serum HER2. Overall anti-HER2 therapy was well tolerated; however 2 patients receiving lapatinib and one receiving trastuzumab plus lapatinib stopped treatment early due diarrhoea and fatigue. Kurbacher *et al.* Abstract 20P.

Practice point and future research opportunities

These findings confirm results from a previous study of trastuzumab-based therapy in HER2-negative patients with elevated serum HER2-positive values and suggest that these patients may be candidates for HER2 targeted therapies. This study is limited by a small sample size and results from randomised phase III trials in patients with occult HER2-positive metastatic breast cancer are needed.

GENOMICS AND PROTEOMIC ANALYSES OF BREAST CANCER

Potential biomarkers for response in HER2 non-amplified patients participating in the GeparQuattro and GeparQuinto trials

Stefanie Avril, Department of Pathology, Technische Universität München, Munich, Germany, presented findings from a study that suggested novel biomarkers for response to anti-HER2 treatment in combination with chemotherapy in patients with HER2-positive breast cancer that do not show HER2 amplification. This study enrolled 98 out of 1060 patients that were HER2 non-amplified by central review to test whether phosphorylated HER2 (pHER2) and co-activation of downstream targets could be predictive of response to anti-HER2 treatment in these patients. Patients were also classified as HER2-positive (IHC, FISH) by local testing and were participating in the GeparQuattro and GeparQuinto trials where they received neoadjuvant anti-HER2 treatment consisting of trastuzumab or lapatinib in combination with anthracycline-taxane-based chemotherapy. The investigators evaluated the potential benefit from anti-HER2 treatment in these patients by assessing the levels of pHER2 and downstream targets including pHER3, EGFR, AKT, PI3K, ERK, PTEN, S6RP together with the phosphorylated forms using reverse-phase protein arrays.

Histopathological complete response (pCR; ypT0/is) was achieved by 25 (26%) patients; however, levels of pHER2 were not significantly different between groups of histopathologic responders and non-responders. S6 ribosomal protein (S6RP) and phosphorylated S6RP (pS6RP) were the only proteins that significantly associated with pCR ($p = 0.01$ and $p = 0.03$, respectively). Higher expression of these proteins was observed prior to treatment and low expression of S6RP and pS6RP associated with prolonged disease free ($p < 0.01$) and overall survival ($p < 0.05$). Expression levels of S6RP and pS6RP did not associate with other HER2 signalling proteins, all of which showed a positive correlation with each other.

Expression of S6RP, and pS6RP showed a significant association with short and long-term outcome following anti-HER2 treatment in HER2 non-amplified breast cancer patients but the activation status of the HER2 pathway, as reflected by pHER2 and other downstream targets, was not predictive of response, and showed no association with S6RP expression. The authors' conclusion was that this suggests S6RP and pS6RP may be novel predictive biomarkers for response to anti-HER2 treatment in non-amplified patients that may act independently of global HER2 pathway activation. Avril *et al.* Abstract 24P**.**

Practice point and future research opportunities

Expression of S6RP, and pS6RP was decreased following anti-HER2 treatment in HER2 non-amplified breast cancer patients and lower expression of these proteins showed a significant association with disease free- and overall survival. S6RP and pS6RP expression may be a new indicator of outcome following anti-HER2 treatment in this cohort.

Detection of HER2-status and genomic analysis of disseminated cancer cells of non-metastatic breast cancer patients with HER2-negative and positive primary tumours

Elisabeth Doblinger, Experimental Medicine and Therapy Research, University of Regensburg, Regensburg, Germany, and colleagues developed a quantitative real-time PCR (qPCR) method following Ampli1™ single cell whole genome amplification (WGA) to determine the HER2-status of disseminated cancer cells (DCCs) from breast cancer patients with HER2-negative and HER2-positive primary tumours. They then determined whether the primary tumours and DCCs showed similar or disparate levels of HER2 amplification. The DNA of isolated DCCs was amplified using the Ampli1™ WGA kit. HER2 amplification was assessed by qPCR. Selected DCCs were subsequently analysed for genome-wide copy number alterations.

The study enrolled 56 breast cancer patients who had no evidence of metastasis; 85 cytokeratin-positive cells were isolated from the bone marrow of these patients. Of these, 70 cells were from 45 patients with HER2-negative primary tumours and 15 cells were obtained from 11 patients with HER2-positive primary tumours.

Of patients with HER2-negative primary tumours, HER2-positive DCCs were isolated from 7 patients; therefore, the disparity for HER2 status was 18%. One HER2-positive DCC was detected out of 9 patients with HER2-positive primary tumours, yielding a disparity rate of 89%. In all cases having HER2-positive DCCs, additional DCCs were HER2-negative. Comparative genomic hybridization analysis revealed that HER2-positive DCCs rarely displayed losses and gains; furthermore, all losses and gains detected were small. Doblinger *et al.* Abstract 25P.

Practice point and future research opportunities

These findings show that the disparity rate for HER2-status between disseminated cancer cells and the respective HER2-positive primary tumours is higher than that detected between these cells and their HER2-negative primary tumours. Since HER2-positive disseminated cancer cells showed few and small genomic alterations, HER2 amplification may represent an early, but not initiating, event in the genomic evolution of these cells.

Whole exome sequencing of circulating and disseminated tumour cells in patients with metastatic breast cancer

Dieter Peeters, Centre for Oncological Research, University of Antwerp, Antwerp, Belgium, discussed their study, which assessed the potential of circulating tumour cells (CTCs) to provide a repeatedly accessible source of tumour cells for the real-time assessment of tumour characteristics. This study aimed to determine the degree of molecular heterogeneity existing within a CTC population and the extent to which CTCs actually reflect the mutational profiles of metastasis. This study compared the mutation profiles of CTCs and synchronously isolated disseminated tumour cells (DTC) from metastases of patients with clinically progressive metastatic breast cancer. CTCs isolated from 7.5 ml blood samples via enrichment by the

CellSearch system were purified and sorted into several batches of 1-125 CTCs using the DEPArray system. DNA was isolated and amplified using the Ampli1 whole genome amplification kit and subjected to whole exome paired-end sequencing. DTCs from metastatic effusions, tissue from solid metastases or primary tumour, or bulk CTCs from patients having >10.000/7.5 ml (CellSearch Profile), are being sequenced as a comparator for mutation profiles. Leukocyte DNA was sequenced for somatic mutation analysis.

Until the date of presentation, 8 samples of CTCs and a CellSearch Profile from patient 1 and 4 samples of CTCs, 2 samples of DTC from a pleural effusion, and the primary tumour from patient 2 have been sequenced. The average base coverage was 13.6x in patient 1 and 11.8x in patient 2 for CTC/DTC samples, 175x for the CellSearch Profile, and 120x for the primary tumour sample. A total of 29.6% to 53.6% of the exomes of amplified products were uncovered, probably due to technical limitations of the WGA procedure.

In patient one, good concordances were seen for variants identified with MuTect in 28 frequently mutated genes between the CTC and the CellSearch Profile samples. In patient two, the same H1047R PIK3CA mutation was identified in the primary tumour and in all CTC/DTC samples. In-depth analyses of the full exome data are ongoing. Peeters *et al.* Abstract 26P

Practice point and future research opportunities

Findings from this analysis could provide useful information regarding the extent of molecular heterogeneity that exists within CTC populations and between CTC, the primary tumour and disseminated tumour cells.

Unique microRNA profile identified in breast cancer diagnosed in very young women

Gloria Ribas, Department of Haematology and Medical Oncology, Biomedical Research Institute INCLIVA. Hospital Clinico Universitario de Valencia, Valencia, Spain and colleagues compared panels of microRNA data from women diagnosed with cancer aged 35 and younger versus women aged 65 and older to answer the question of whether breast cancer in very young (BCVY) women arises from the deregulation of different underlying mechanisms, something that could make this disease an entity separate and unique from breast cancer diagnosed in older patients. Prof. Ribas presented data from a validation study of their previous work. This study analysed a publically available microRNA dataset of 1302 breast tumours from Cambridge Breast Unit under European Genome Archive (www.ebi.ac.uk/ega) with EBI access EGAD00010000438.

Data were stratified according to patient age, which yielded a BCVY cohort of data from 33 patients aged 35 and less and an older cohort comprising data from 712 patients aged 65 and greater with breast cancer. The investigators used microRNA as a biomarker in analysing the relevant gene targets and pathways with GO Ontology and Reactome methods.

Findings showed a differential and unique microRNA expression profile between the cohorts, with 56 of the 853 microRNAs analysed being deregulated in BCVY women. Of the 56 microRNAs deregulated in BCVY, 31 microRNAS were upregulated and 25 were downregulated. In the upregulated microRNA category, 9 (29 %) belong to the polycluster miR-17/92, which includes among gene targets the regulatory molecules PTEN, and E2F1-3 family proteins, including TGF-b, Smad2-4, and BCL2L11. The other 7 (22%) upregulated microRNAs belong to the microRNA-515 family. The miR-17/92 and microRNA-515 families represent just above 50% of the total group. In the group of 25 downregulated microRNAs, the authors highlighted the mir-29 y mir-30 families, which comprised 28% of the group. These families contain miR-29c*, miR-29c, miR29b, and miR-30a*, miR-30c, miR-30b, and miR30e, which correspond to gene targets that include CDK6 and IGF1R.

The authors concluded that the microRNAs found to be deregulated in BCVY affect the cell cycle, thereby potentially altering apoptosis and disease metastasis. Although the individual miR profiles do not fully coincide with original results from the previous study, the signalling pathways altered are convergent. Ribas *et al.* Abstract 30P

Practice point and future research opportunities

A distinct microRNA signature was determined for the subtype of breast cancer diagnosed in very young women that is a separate and distinct molecular signature from that seen in older women with breast cancer, which may facilitate further research into the underlying mechanism that may help to identify therapeutic targets in breast cancer in very young women.

Multigene sequencing for the genetic diagnostics of patients with early-onset or familial breast cancer

Lead investigator Po-Han Lin, Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan, pointed out that epidemiologic studies have established that the median age of breast cancer patients is younger in Taiwan than in western populations, and that breast cancer patients in Taiwan have a greater risk of and a shorter interval to developing contralateral breast cancer. Noting that this suggests genetic predisposing factors, the investigators carried out this study using a customised multigene sequencing panel to investigate the genetic characteristics of patients with early-onset breast cancer or having a significant family history of breast cancer in Taiwan. The study recruited 106 patients with either early-onset breast cancer that was diagnosed at less than 35 years of age or bilateral breast cancer, or patients experiencing breast cancer onset before age 50 years plus at least one first or second-degree relative with breast or ovarian cancer, or patients diagnosed with breast cancer after age 50 but having 3 relatives with breast or ovarian cancer. Germline genomic DNA for next-generation sequencing was extracted from mononuclear cells in the peripheral blood.

Germline pathogenic mutations of known cancer susceptibility genes were found in 24 (22.6%) patients. The mutations included one ATM, 8 BRCA1, 10 BRCA2, one BRIP1, one FANCI, one MSH2, one RAD50 and one TP53. The mutation prevalence was 22.9% in women aged <35 years, 22.0% in women aged between 35 to 50 years, and 25.0% in women aged 50 years and

older. When assessed according to the molecular subtype of breast cancer, the mutation prevalence was 17.6% in patients with hormone receptor positive, human epidermal growth factor receptor 2 negative (HR-positive HER2-negative) breast cancer, 16.7% in HR-positive HER2-positive breast cancer, 60.0% in triple-negative breast cancer (TNBC), and no mutations were detected in tumours that were only HER2-posiive ($p = 0.003$). Patients with TNBC showed the most BRCA mutations with 7 of 8 BRCA1 mutations and 2 of 10 BRCA2 mutations detected in this cohort. Other BRCA1 and BRCA2 mutations were detected in patients with HR-positive HER2-negative tumours. ATM, RAD50 and TP53 mutations were detected in patients with HR-positive HER2-positive tumours, BRIP1 and MSH2 mutation were seen in patients with HR-positive HER2-negative tumours, and FANCI mutation was detected patients with TNBC. Lin *et al.* Abstract 31P

Practice point and future research opportunities

These findings reveal a 22.6% mutation rate overall in patients with familial or early-onset breast cancer in Taiwan and a significantly higher mutation rate in TNBC than either HR-positive or HER2-positive tumours. This contributes to the body of information that enables cancer risk assessment, genetic counselling and targeted cancer treatment.

Genetic alterations in primary breast cancer associate with brain metastases

Harriet Wikman, Tumour Biology, UKE Universitätsklinikum Hamburg-Eppendorf KMTZ, Hamburg, Germany, and colleagues screened both primary and metastatic breast tumours by gene expression and copy number profiling to identify potential new markers and treatment targets in brain metastases. Immunohistochemistry was done on brain metastases tissue microarrays (TMAs) to confirm pathway alterations identified by profiling and the blood from patients with brain metastases was screened for the presence of circulating tumour cell (CTCs).

The investigators identified genetic alterations in the primary breast tumours that associated with metastatic spread to the brain. They found that two major pathways, the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2), were implicated in driving brain metastasis. Either a gain of EGFR or a loss of phosphatase and tensin homologue (PTEN) was detected in 91% of patients with brain metastases who had a triple-negative (TNBC) tumour. However, amplification of HER2 with concurrent EGFR alteration was rare and detected in just 5.7% of all brain metastases. EGFR alterations were found in only 7% of hormone receptor (HR) positive tumours; in contrast, loss of PTEN was detected in 23% of HR positive tumours. These markers may be useful in the identification and subclassification of CTCs from patients, where EGFR and HER2 expression seem to more heterogeneous, according to the authors who are currently characterising CTCs obtained from these patients. Wikman *et al.* Abstract 32P

Practice point and future research opportunities

In the patient cohort studied, almost all patients with brain metastases showed alterations in the HER2/EGFR signalling pathway caused by partially mutually exclusive alterations**.**

Genetic study of triple negative breast cancers reveals prognostic factors for outcome

Lead investigator Meriem Boukerroucha, Human Genetic, University of Liege, CHU Sart Tilman, Liege, Belgium presented findings from a study done to elucidate the correlation between BRCA1-related molecular parameters, other tumour characteristics and clinical follow-up in order to better classify triple negative breast cancers (TNBC) and define prognostic factors.

The loss of BRCA1 expression plays a key role in TNBC, where it may undergo germinal mutation followed by deletion of the second allele, or demonstrate negative regulations by promoter methylation or miRNA-mediated silencing. While TNBC does not respond to endocrine therapy or trastuzumab, BRCA1-deficient tumours have defective DNA repair making them sensitive to DNA-damaging chemotherapy or PARP inhibitors; therefore, identification and characterisation of BRCA1 may be useful in guiding treatment.

Using tumour tissue from 60 patients with TNBC, the investigators quantified BRCA1 protein and mRNA expression in situ, and established the methylation status of the promoter. They also investigated cytokeratin (CK) 5 and 6 expression, and the relationship between BRCA1 and its interacting protein, BARD1. The investigators also quantified the expression of 29 microRNAs previously reported to be correlated with survival in breast cancer. This study used one miRNA, miR-548c that is expressed on tumours plus 3 prognostic factors that included tumour size, lymph node invasion and CK 5/6 expression status to predict for relapse by logistic regression with an area under the curve (AUC)= 0.96 and a 95% confidence interval (CI) 0.883, 1.

Using just the 3 prognostic factors, tumour size, lymph node invasion and CK 5/6 expression status, relapse prediction could be done with an AUC = 0.75 (95% CI 0.6, 0.91); however, when the BRCA1 mRNA and protein levels plus miR-210 and miR-548c tumoural expression were added to the prognostic factors, the ability to predict relapse was improved to AUC = 0.82; 95% CI 0.67, 0.96. Boukerroucha *et al.* Abstract 33P

Practice point and future research opportunities

Tumoural miR-548c expression improved the ability to predict relapse generated by 3 known prognostic factors in this study. miR-548c may be a potential novel prognostic factor of relapse in patients with TNBC that needs confirmation.

PIK3/AKT signalling pathway is most often altered in breast cancer

Findings from a large series of genomic profiling were presented by Juan Miguel Cejalvo, and colleagues at the Hospital Clinico Universitario de Valencia in Valencia, Spain, that showed the PIK3/AKT signalling pathway was more often deregulated and had more genetic mutations than the MAPK pathway in both primary and metastatic breast cancer tumours. The team used high-throughput genomic profiling to evaluate the frequency at which the PI3K/AKT and MAPK signalling pathways are deregulated in breast cancer to identify frequently occurring oncogene alterations that could be targeted. This evaluation was carried out using primary and/or metastatic tumour samples from 214 patients with metastatic breast cancer that were treated at the Hospital Clínico of Valencia to determine the incidence of mutations in genes known to harbour mutations important in cancer, including PIK3CA, AKT1, KRAS, NRAS and BRAF. The genetic assessments were done by MassARRAY technology, which allows the simultaneous analysis of multiple classes of genetic markers and the levels of PTEN expression were determined by immunohistochemistry done on formalin-fixed paraffin embedded (FFPE) samples.

Genomic alterations occurred most often in the PI3K/AKT pathway in the breast cancer samples evaluated in this series. PIK3 upregulation was the most commonly occurring genomic alteration; PIK3 was up-regulated in 46% of primary tumours and 26.9% of metastatic tumours. AKT was upregulated to a far lesser degree in 2.78% of primary and 6.86% of metastatic tumour samples. Decreased PTEN levels were also detected in 27.91% of primary and in 38.1% of metastatic tumour samples tested. Fewer mutations in the MAPK pathway were detected; the frequency of genomic alteration in primary and metastatic tumours, respectively, was 14.29% and 11.88% for KRAS, 2.86% and 6.93% for NRAS and 2.63% and 4.39% for BRAF.

E542K was the most often detected mutation in primary tumours, whereas H1047R was the most frequently occurring mutation in metastatic tumour samples. In 22 primary tumour samples, the PI3K mutations most frequently detected were E542K, which occurred in 40.91% of samples, E545K in 31.82%, H1047R in 18.18%, C420R in 4.55%, and Arg886Gln mutations, which were seen in 4.55% of primary tumour samples. In 32 metastatic tumour samples, PIK3 alterations that were detected included H1047R in 42.86% of samples, E545K in 25.71%, E542K in 17.14%, Arg88Gln in 5.71%, M1043I in 5.71% of samples, and Asn345Lys was altered in 2.86% of specimens.

Genetic alteration patterns were specific to tumour type; the highest frequency of mutation in the PI3K/AKT pathway and the highest degree of PTEN loss were observed in triple negative breast cancer (TNBC) wherein PTEN loss was detected in 80% of primary and 60% of metastatic tumour samples; KRAS mutations were also detected in 33.3% and 7.14% of primary and metastatic TNBC tumours, respectively. Cejalvo *et al.* Abstract 36P

Practice point and future research opportunities

In this series, a higher incidence of breast cancer oncogenes in the PI3K/AKT than in the MAPK signalling pathway was observed. MAPK mutations were infrequent in both primary and metastatic breast cancer tumours compared to the PI3K-AKT pathway, which was commonly altered with PI3K mutations being the most relevant. Strategies of novel drug development may find more drugable targets within the PI3K/AKT signalling pathway.

BIOMARKERS IN BREAST CANCER (PROGNOSTIC, PREDICTIVE, AND PHARMACODYNAMIC)

Guiding breast cancer treatment using a biomarker for bevacizumab response demonstrates cost effectiveness

Patricia Blank, European Centre of Pharmaceutical Medicine (ECPM), University of Basel Institute of Pharmaceutical Medicine, Basel, Switzerland and colleagues in German and Sweden evaluated the cost-effectiveness of testing breast cancer patients for levels of vascular endothelial growth factor (VEGF), as a predictor for response to bevacizumab, to guide whether adding it to chemotherapy regimens could ultimately result in increased quality adjusted life-years (QALYs) for patients with hormone receptor (oestrogen or progesterone) positive (HR-positive) human epidermal growth factor receptor 2 negative (HER2-negative) breast cancer. Overall, patients with HER2-negative breast cancer have shown only modest benefit from neo-adjuvant bevacizumab, leading the investigators to try to determine the health economic impact as well as the incremental cost-effectiveness ratio (ICER) of VEGF-A guided use of bevacizumab using a life-long Markov state transition model. They compared 6 alternative strategies that comprised four different VEGF-A cut-off values, plus 2 strategies that evaluated the use of neoadjuvant bevacizumab in either all or no patients as reference. Data regarding overall and metastasis-free survival of 830 women participating in the GeparQuinto trial were used and effectiveness was assessed as QALYs. The treatment cost was given in EURO at the year 2013 value and was assessed from a German third-party payer perspective.

Model projections placed costs per patient in a range from EURO 37,042 for the reference strategy of no adjunct bevacizumab to double that cost of EURO 78,367 when bevacizumab was added to all treatment regimens, the second reference strategy.

Not adding bevacizumab to chemotherapy treatment yielded 14.031 QALYs per patient.

However, QALYs were increased with the use of VEGF-A guided strategies from 14.220 with the bevacizumab dose cut-off of 450 pg/mL to 14.235 QALYs with a cut-off 339 pg/mL. The preferred strategy was a VEGF-A guided strategy at a bevacizumab dose cut-off of 450 pg/mL, which increased QALYs by 0.189 per patient at an additional cost of 11,191 EURO yielding an ICER of 59,161 EURO per QALY. Blank *et al.* Abstract 45P.

Practice point and future research opportunities

These findings suggest that VEGF-A testing may be sensibly used to guide the addition of bevacizumab to chemotherapy in patients with HR-positive HER2-negative breast cancer. Using Germany as an example, a cut-off bevacizumab dose of 450 pg/mL might be the most cost-effective while increasing QALYs per patient.

p53 is mutated in the majority of triple negative breast cancers but shows no association with pathological complete response; analysis of data from the GeparSixto trial

According to Silvia Darb-Esfahani, Institute of Pathology, Charite Berlin Mitte, Berlin, Germany, p53 is known to be among the most frequently mutated genes in breast cancer, but it is unclear whether it may be an indicator for response in different cancer subtypes and the predictive value regarding response to chemotherapy has also not been defined. She noted that the CALGB 40601 study recently demonstrated that p53 mutation status had a positive impact on response to neoadjuvant therapy with anthracyclines, taxanes and anti-HER2 agents in patients with HER-positive breast cancers. To further elucidate these findings, Prof. Darb-Esfahani and colleagues performed Sanger sequencing on 450 core biopsies obtained at baseline from 346 (54.7%) patients with triple-negative and 201 (45.3%) patients with HER2-positive breast cancer prior to receiving treatment in the neoadjuvant phase II GeparSixto clinical trial.

The GeparSixto study evaluated the benefit of adding carboplatin to paclitaxel plus pegylated liposomal doxorubicin given as a weekly regimen for 18 weeks in 595 patients with breast cancer. GeparSixto showed significantly improved pathologic complete response (pCR) in the TNBC subgroup, wherein pCR was achieved by 37.9% of the control arm versus 58.7% of the carboplatin arm ($p < 0.05$). However, pCR was achieved by 36.3% and 33.1% of control versus carboplatin groups, respectively, in HER2-positive patients.

In the study presented at IMPAKT, sequencing specific to the DNA-binding domain of p53 in exons 5 to 8 revealed p53 mutations in 66.0% of the entire cohort that were evenly distributed between exons 5 to 8 at 22.6% and 26.6%, respectively. Significant associations were seen between mutated p53 and disease subtypes; p53 was mutated in 74.8% of TNBC tumours compared to 55.4% of HER2-positive tumours ($p < 0.0001$). Tumours containing p53 mutations also had significantly higher ki67 indices ($p = 0.003$), but no significant association between p53 mutation status within the HER-positive or TNBC groups and ki67 indices was observed ($p > 0.05$). In patients with mutated p53, pCR, defined as ypT0 ypN0 or absence of invasive cancer and in-situ cancer in the breast and axillary nodes, was achieved by 38.0% of patients with TNBC compared to 37.9% of patients with HER2-positive tumours, odds ratio (OR) 1.01, 95% confidence interval (CI) 0.67, 1.50 ($p = 0.977$).

No association was seen between p53 status and pCR or within the groups of TNBC and HER2-positive tumours. The pCR rate was 56.5% in patients with TNBC plus mutated p53 compared to 58.1% in patients with TNBC and wildtype p53. The pCR rates in HER-positive and mutated p53 compared to HER-positive wild-type p53 were 70.8% versus 64.8%, respectively. In addition, when comparing p53 mutated and wildtype, no significant associations were seen with age, histotype, expression levels of the oestrogen or progesterone receptors, PIK3CA mutations or the number of tumours infiltrating lymphocytes ($p > 0.05$). Darb-Esfahani *et al.* Abstract 46P

In contrast to the CALGB 40601 data, this study found no correlation between p53 mutations and pCR after comparable neoadjuvant chemotherapy. Also p53 mutations were not linked to clinico-pathological parametres, immunological features or to PIK3CA mutations. Although p53 was found to be mutated in nearly half of human epidermal growth factor receptor 2 positive (HER2-positive) breast cancer and just under three quarters of triple negative breast cancer (TNBC), no association could be seen between expression of the mutated form and patient response to neoadjuvant therapy. More study is needed to reconcile these reports and to elucidate the role of mutated p53 in breast cancer.

Defining prognostic and therapeutic selective classes of TNBC

Lead Investigator Erik Knudsen, University of Texas Southwestern Medical Centre at Dallas, Dallas, USA headed a team that used an integrated combination of biomarker analyses and targeted drug screening to define actionable subtypes of triple negative breast cancer (TNBC) for development of much needed treatment for this disease.

The investigators analysed survival data from 218 women with TNBC for the expression of multiple markers identified by immunohistochemistry (IHC). The markers focused upon were associated with either immune function, such as CD163, the cell cycle, RB, and defined subtypes, for example vimentin and the androgen receptor (AR). Using affinity propagation clustering and random forest approaches they were able to define specific subtypes of TNBC that were highly enriched for prognosis of better survival. Models mimicking these subtypes were evaluated for sensitivity to known cancer drugs using high-throughput approaches and in selected combinations based on the marker profile.

Upon analysis using unsupervised methods, classes of TNBC defined by IHC were found to be associated with both preferred and poorer prognosis, and these associations remained significant in multivariate analysis. Tumours having combined loss of RB and PTEN associated with a 5-year survival of >85% ($p < 0.01$), whereas mesenchymal tumours that expressed vimentin associated with a prognosis of 5-year survival of <25% ($p < 0.005$).

The investigators also looked at two exclusive classes of TNBC, RB-negative and AR-positive, which represent approximately 50% of cases and evaluated these classes for therapeutic sensitivities. RNA sequencing of clinical RB-negative AR positive cases revealed that tumours deficient in RB express high levels of genes that activate CDK5, PLK1, AURK, CHK kinases, and they found that RB-deficient tumour models were selectively sensitive to agents targeting these kinases. In contrast, AR positive tumours retain RB activity and exhibit a low proliferation index in clinical cases. TNBC models of AR positive disease were particularly sensitive to CDK4/6 inhibitors alone and in combination with AR-antagonists. Knudsen *et al.* Abstract 47P.

Practice point and future research opportunities

These data indicate that using a limited collection of markers it is possible to delineate prognostic subtypes of TNBC and, that within TNBC cases, tumours with specific genetic alterations have distinct vulnerabilities that can be targeted therapeutically.

Improved concordance rates for local and central HER2 status

According to Berit Pfitzner, Charité Universitätsmedizin Berlin, Berlin, Germany who presented results on behalf of the German Breast Group, inter-laboratory tests have been performed in Germany since 2000 to preserve quality in breast cancer diagnosis and participation became mandatory for certification of breast cancer centres in 2007. Prof. Pfitzner presented findings from an analysis of a very large dataset from 5 multicentre clinical trials of neoadjuvant treatment of patients with breast cancer that shows a steady decrease of the discordance rate comparing centrally and locally assessed human epidermal growth factor receptor 2 (HER2) status on breast cancer tumours.

The investigators compared the centrally and locally assessed HER2 status of 1597 patients with HER2 positive cancers participating in the Gepar-Trio, Quatro, Quinto, Sixto and Septo trials between 2001 and 2013 to determine whether the rate of discordance between central and local HER2 status had decreased over the past years, since discordant rates of approximately 20% to 30% in HER2 had been reported in several recent retrospectives studies. This range of discordance has been repeatedly demonstrated despite differences in methodology of assessment for the biomarker between the primary and relapsed lesions, inclusion of both local and regional relapses with the distant tumours, and heterogeneity in patient populations.

They found the discordance rate decreased from the earliest to most recent trials, from 52.4% in the GeparTrio trial to 8.4% in the GeparSepto trial. Discordance rates for the other trials showed a steady decline; the discordance rate was 25.4% in GeparQuattro, 22.7% in GeparQuinto, and 7.0% in the GeparSixto trials. Regarding the 62.6% of patients with hormone receptor positive, HER2-positive tumours, the HER2 discordance rates were 58.8, 30.8%, 29.2%, 0, and 9.2% in the Gepar Trio, Quattro, Quinto, Sixto and Septo trials, respectively. The discordance rate for the 37.4% of patients with hormone receptor negative, HER2-positive tumours also showed a linear decrease, except in the GeparSixto trial wherein the discordance rate was 16.3%. The rate in the other trials was highest in GeparTrio at 37.9% and decreased steadily to 18.9% in GeparQuattro, 13.9% in GeparQuinto and finally to 6.1% in GeparSepto. Pfitzner *et al.* Abstract 58P

Practice point and future research opportunities

Findings from this analysis of a large dataset from 5 neoadjuvant breast cancer studies show a steady decrease in the discordance rate comparing the central and locally tested HER2 status. Detection of HER2 overexpression or amplification is essential for therapeutic management of breast cancer patients; HER2 amplification and overexpression of HER2 occurs in approximately 18% to 25% of invasive human breast cancers. HER2 serves as a therapeutic

target for several antibodies and determination of HER2 expression can indicate if a patient will respond to them.

AP-2gamma tumour expression is a biomarker for resistance to endocrine therapy in patients with metastatic breast cancer

Luca Malorni, Hospital of Prato, Istituto Toscano Tumori, Prato, Italy, headed an international team that had recently concluded the TransCONFIRM study, which was an analysis of samples from patients with metastatic breast cancer treated with fulvestrant given at either 500 mg or 250 mg. Findings from TransCONFIRM showed that AP-2gamma is differentially expressed between responders and non-responders to fulvestrant.

The study reported at IMPAKT tested the prognostic role of AP-2gamma, a transcription factor implicated in breast cancer, as a predictive marker for response to fulvestrant. AP-2gamma levels were characterized by immunohistochemistry on tissue microarrays of 124 primary breast cancer samples from patients in the TransCONFIRM cohort and also from a separate cohort of primary breast cancer samples from an institutional tissue bank. The level of AP-2gamma levels in the nucleus, plus levels of ki-67 and HER2 status were reviewed centrally.

Patients having primary tumours expressing AP-2gamma experienced progression-free survival nearly half of that seen in similar patients having negative tumours after fulvestrant treatment for metastatic breast cancer. In addition, high expression of AP-2gamma was shown to associate with poorer outcome following endocrine therapy. Analysis of survival data showed that patients in the TransCONFIRM cohort with AP-2gamma positive tumours experienced significantly shorter progression free survival (PFS) compared to patients with negative tumours; median PFS was 5.4 months in 61 patients with AP-2gamma positive tumours versus 9.6 months in patients negative for AP-2gamma, hazard ratio (HR) 1.082; 95% confidence interval (CI) 1.013, 1.155 ($p = 0.02$).

Samples were stratified per molecular subtypes according to the St. Gallen definition; 29% of 51 luminal A samples, 62% of 50 of luminal B/HER2-negative samples, 90% of 10 the luminal B/HER2-positive subtype, 36% of 11 of the triple negative, and 100% of 2 HER2-positive subtype samples scored positive for AP-2gamma. Analysis of data from the subgroup of 103 luminal A/B samples also showed a similar trend of poorer survival in patients with tumours expressing AP-2gamma that did not reach statistical significance ($p = 0.05$).

AP-2gamma positivity occurred more frequently in higher grade tumours and in tumours that expressed markers for cell proliferation, both predictors of poorer prognosis; 76% of grade 3 compared to 26.7% of grade 1 tumours were positive for AP-2gamma, as were 66% of tumours with high Ki-67 versus 32% with low levels of Ki-67. An identical analysis of an independent cohort of samples from an institutional tissue bank confirming AP-gamma as a predictive biomarker for fulvestrant was ongoing at time of abstract submission and will be reported at a later date. Malorni *et al.* Abstract 60P

Practice point and future research opportunities

These findings from a molecular analysis of metastatic breast cancer samples from a cohort of patients and a cohort of samples from an institutional tumour registry establish AP-2gamma as a predictive biomarker for endocrine resistance. Increased expression of AP-2gamma in primary tumour tissue samples associated with shorter PFS when treated with fulvestrant in the metastatic setting; PFS in patients with primary tumours expressing high levels of AP-2gamma was nearly half of that seen in similar patients having negative tumours after fulvestrant. Additional studies are warranted to further assess the prognostic role of AP-2gamma for hormone receptor positive metastatic breast cancer patients treated with different endocrine agents and to determine the mechanism behind AP-2gamma endocrine resistance.

Diverse molecular characteristics in pregnancy associated breast cancer

Zorica Tomasevic, Daily Chemotherapy Hospital, Institute of Oncology and Radiology of Serbia, Belgrade, Serbia and colleagues defined the molecular characteristics of pregnancy associated breast cancer (PABC) that was diagnosed in patients aged 40 years and less within 36 months of pregnancy. The personal and familiar anamnesis for all malignancies (FAM) for each patient was also investigated.

The study prospectively enrolled 39 patients with confirmed invasive breast cancer from January 2012 to December 2014. The median patient age was 31 (range: 24 to 40) years, and the median time from pregnancy was 20 (range: 20 to 36) months. Ten (25.6%) patients were diagnosed during, or within 3 months of pregnancy, and 2 (5.1%) patients were treated for Hodgkin disease in adolescence, 14 and 18 years prior to being diagnosed with PABC. FAM was known for 28 (70%) patients and revealed that 8 of 16 patients with positive FAM had one or more relatives with breast or ovarian cancer.

More than half, 58.4%, of patients had ductal carcinoma, 20.5% had lobular carcinoma, 15.3% of patients had inflammatory breast cancer with confirmed lymphangiosis, and 5.1% of patients had other histology types. At diagnosis of PABC, 18 (46.1%) patients presented with stage I or II disease, 17 (43.5%) with stage III, and 4 (10.2%) patients had stage IV disease.

Tumour subtypes were diverse among these patients: molecular PABC characteristics included human epidermal growth factor receptor 2 positive (HER2-positive) in 28.2% of patients, oestrogen receptor positive HER2-negative (HR-positive HER2-negative) in 41.0% of patients, and 30.7% of patients had triple negative breast cancer. Tomasevic *et al.* Abstract 66P

Practice point and future research opportunities

Further characterisation of cancer subtypes is warranted in patients with pregnancy associated breast cancer. This small cohort revealed a higher incidence of triple negative and HER2-positive types than in the general population, as well as a higher incidence of inflammatory breast cancer. Although pregnancy is considered a protective factor for the development of breast cancer, women with a history of juvenile and adolescent cancer and/or close relatives with breast or ovarian cancer should be monitored for breast cancer during pregnancy.

Varied outcome observed in tumour subtypes based on receptors expressed in males with breast cancer

Siddhartha Yadav, Beaumont Health System, Royal Oak, USA, reported that the outcome in male patients with breast cancer following treatment varies according to tumour subtypes based upon receptor expression. His team characterised the tumour subtypes found in a large cohort of males with breast cancer according to the different receptors expressed on these tumours. Their findings underscored the importance of molecular testing in these patients and suggested that males with breast cancer can also benefit from targeted therapies aimed at these receptors. Breast cancer in males is a rare disease with an incidence of approximately 1% among all breast cancers, which possibly accounts for the current paucity of molecular data for this cohort, according to Dr. Yadav.

Data from 922 patients with known receptor subtypes included in the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) programme for all male breast cancers diagnosed since 2010 were used for this analysis and revealed that most tumours from male breast cancer patients express a hormone receptor; 757 (82%) patients had tumours that were hormone receptor positive and human epidermal growth factor receptor 2 negative (HR-positive HER2-negative), 135 (15%) patients were HR-positive HER2-positive, 11 (1%) patients were HR-negative/HER2-positive, and 19 (2%) patients had triple-negative (HR-negative HER2-negative) breast cancers. Across the different subtypes of male breast cancers, no differences were recorded for racial composition or histology. However, differences across subtypes regarding the overall stage ($p = 0.052$) and nodal status ($p = 0.051$) were observed. According to the authors, significant differences between cohorts stratified by the receptor subtype were seen in histologic grade, tumour stage and metastasis status at diagnosis.

Poorer one-year survival rates were seen with TNBC, which was diagnosed in patients at a younger age. One-year overall survival (OS) rates were improved in patients with tumours expressing at least one receptor over patients with TNBC. The one-year OS rate in male breast cancer patients was 95% overall; however, a breakdown by subtype revealed a rate of 96.7% in the HR-positive HER2-negative subtype, 90.0% in the HR-negative HER2-positive subtype and 89.9% in the HR-positive HER2-positive subtype compared to 67.9% in the HR-negative HER2-negative (TNBC) subtype. A similar pattern was seen for one-year cause specific survival where rates were 96.6% overall, 98.2% for the HR-positive HER2-negative subtype, 90.0% in the HR-negative HER2-positive subtype and 92.2% in the HR-positive HER2-positive subtype versus 67.9% in patients with TNBC. Yadav *et al.* Abstract 72P

Practice point and future research opportunities

Significant differences were observed in response to treatment according to tumour characteristics between different subtypes of male breast cancers. The strongest example was seen in patients with triple-negative breast cancers, where diagnosis was likely to be made at a younger age and patients also demonstrated poorer one-year survival. Additional research of breast cancer in males is needed to better characterise the role of receptor subtypes in tumour pathology and outcome to optimize treatment for these patients.

Standardised uptake value of 18F-FDG-PET-CT is in accordance with the 21-gene recurrence score (Oncotype Dx) in patients with ER-positive and HER2-negative breast cancer

Sung Gwe Ahn, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea and colleagues investigated up-regulated glucose uptake, which is associated with a high proliferation rate and increased metabolic activity in breast cancer. The team had previously provided evidence that tumours with elevated glucose uptake carry a higher risk of recurrence in oestrogen receptor positive (ER-positive) breast cancer. In the retrospective study presented at IMPAKT, they investigated the relationship between standardised uptake value (SUV) of 18F-FDG-PET-CT and the risk of recurrence, as determined by the 21-gene assay recurrence score (RS) using the Oncotype DX® Breast Cancer Assay. They identified 105 patients with a RS who had undergone preoperative 18F-FDG-PET-CT and obtained the Maximum SUV of 18F-FDG-PET-CT, using the cut-off point of 4 as in previous studies.

All of the patients had ER-positive and HER2-negative tumours. The 21-gene assay identified 61% of patients as low-risk, 30% as intermediate-risk, and 9% as high-risk. The average RS was 25±13 in the patients with high SUV compared to 14±6 in patients with low SUV ($p < 0.001$). A positive correlation between RS and SUV was found (Pearson' $R = 0.510$, $p < 0.001$), and an inverse correlation between PR score and SUV was also determined (Pearson' $R = -0.415$, $p < 0.001$). However, no correlation of SUV was found with ER or HER2 scores. In 76 patients with low SUV, defined as SUV<4, 54 (72%) patients had low RS. No patients with low SUV had a tumour with high RS. Additionally, 70 (92%) patients had tumours with relatively lower RS of ≤24. All 9 patients with high RS also had a high-SUV tumour. Ahn *et al.* Abstract 73P

Practice point and future research opportunities

Standardised uptake value (SUV) of 18F-FDG-PET-CT demonstrated a positive correlation with the 21-gene recurrence score in ER-positive and HER2-negative patients; patients with SUV lower than 4 were more likely to have lower recurrence scores. These findings provide a novel insight that glucose metabolism has clinical utility in evaluating recurrence in ER-positive and HER2-negative breast cancer.

BREAST CANCER HOST IMMUNE AND STROMAL BIOLOGY

Evaluation of PDL1 expression in breast cancer by immunohistochemistry

A team headed by Cinzia Solinas, Molecular Immunology Unit, Institut Jules Bordet - Université Libre de Bruxelles, Brussels, Belgium tackled the problem of the reliability of PD1/PDL1 detection in breast cancer using immunohistochemistry (IHC). Although PDL1 is a potential predictive biomarker for response to PD1/PDL1-directed cancer therapies, PDL1 detection and characterisation can be problematic due to variation based on tumour heterogeneity, changes in the microenvironment, and technical limitations.

This study aimed to determine the reliability of PDL1 detection by analysing a series of paraffin-embedded tumour samples from 116 untreated patients that were diagnosed between 2001 and 2013. The cohort comprised 44% of women with luminal A, 28% with luminal B, 15% with triple-negative and 15% of patients with HER2-positive breast cancer. The investigators used a double-IHC stain with both a polyclonal and a monoclonal antibody targeting PDL1 (E1L3N clone). They used a second double-IHC stain with antibodies to CD3 and CD20, which are pan T and B cell markers, to determine the relationship between PD1/PDL1 expression and the extent of tumour infiltrating lymphocytes (TIL), and also to characterise their organization in tertiary lymphoid structures (TLS). Membrane PDL1 positivity was defined as the percentage of positive cells among TIL, neoplastic and stromal cells; PDL1-positive was defined as >1% of positive cells and PD1 was considered positive when detected in >5% of TIL. All pathological assessments were done by a well-trained pathologist who was blinded from the clinical data.

Overall, 22% of the tumours were PDL1-positive. The percentage varied according to cancer subtype with 41% of triple-negative, 28% of luminal B, 25% of HER2, and 10% of luminal A tumour samples staining positive for PDL1. It was also determined that PDL1 was more frequently expressed on TILs than other cells; PDL1 expression was seen on 18% of TILs, 7% of neoplastic, and 3% of stromal cells ($p = 0.0002$). PDL1 expression was found to significantly and positively associate with PD1 expression ($p = 0.0005$), Ki67 levels ($p < 0.0001$), the degree of TIL infiltration, ($p = 0.01$) and the presence of tertiary lymphoid structures ($p = 0.006$). Solinas *et al.* 78P

Practice point and future research opportunities

The results of this study indicate that the degree of immune infiltration of a tumour, as reflected by the extent of TIL and their organisation in tertiary lymphoid structures, associates with the expression of potentially targetable immune check-point molecules such as PD1 and PDL1. These findings confirm that they are prognostic immune-biomarkers that may be helpful in identifying patients that could derive clinical benefit from immunotherapy.

MUTATIONS AND RESISTANCE: IMPACT OF MUTATIONS IN THE CLINIC

Pilot study demonstrates feasibility of large-scale molecular screening programme in patients with metastatic breast cancer

Marion Maetens, Breast Cancer Translational Research Laboratory, Institute Jules Bordet, Brussels, Belgium presented findings on behalf of the Breast International Group (BIG) from a pilot study for the AURORA molecular screening programme that aimed to determine the feasibility of large-scale molecular screening, including targeted gene sequencing (TGS) to detect changes in tumour markers in patients with metastatic breast cancer. Molecular characterisation of tumours in breast cancer has historically been done on primary lesions to determine the type of targeted therapy to use in the clinical setting, but growing awareness that metastasis brings with it an evolution of tumour markers and also that tumours become increasingly heterogeneous over time has made the molecular characterisation of tumours throughout the course of disease important to consistently deliver optimal treatment.

Implementing AURORA required preliminary feasibility data and information on the variability of results produced by the various participating laboratories, leading to the initiation of this pilot study that began prior to the launch of the AURORA initiative in 2014. Secondary aims included comparing somatic mutation calls between two TGS platforms and somatic copy number aberrations (SCNA) calls obtained from TGS and single nucleotide polymorphism (SNP) oligonucleotide arrays. Analysis of tumour and normal DNA was done by TGS of cancer-related genes using the Ion Torrent and Illumina platforms. SCNA calls obtained from normalised coverage of TGS data were compared to the output of the ASCAT algorithm on Affymetrix OncoScan formalin fixed paraffin embedded (FFPE) array data.

Four European centres participated in the pilot study, which enrolled 41 patients having one representative metastatic biopsy, a whole blood sample, and less than 10% tumour cellularity. Biopsies were performed in 35 (85%) patients. The metastatic sites most often biopsied included the liver in 43% of patients, followed by the breast in 20% of cases. Biopsies were obtained less frequently from the lymph node and skin in 14% of patients each, the lung in 6%, and bone in 3% of patients each. The tumour cellularity ranged from 10 to 85%.

TGS results were obtained for 26 (74%) of the 35 patients and were reported within a median turnaround time of 9 (range: 5 to 17) working days. The most frequently mutated genes were PIK3CA (50%), TP53 (27%), and ESR1 (15%). Somatic mutations were called in exons covered at >100X based on a fixed threshold of 10% variant allele frequency in the cancer sample. The median number of mutations per patient identified from Ion Torrent was 7 (range: 0 to 29). The overall mutation detection rate was 69% and the “actionable” mutation detection rate was 54%. The copy number alteration (CAN) detection rate was 96%.

Concordance between Ion Torrent and Illumina among mutations for all common target regions was 83% and for clinically “actionable” mutations 100%. However, Ion Torrent has a higher rate of variant detection in terms of higher coverage, false positives, and non-exhaustively.

Concordance analysis between Ion Torrent TGS and SNP arrays is ongoing; in data presented at IMPAKT, concordance of SNVs for all common targets was 51% and 83% after manual

curation. The concordance of potentially actionable SNVs was 78% and 100% after manual curation. Ion Torrent was found to have a higher rate of variant detection. Maetens *et al.* Abstract 92O

Practice point and future research opportunities

Results from this pilot study suggest that the mutation calls validation rate was acceptable, and that Ion Torrent SNVs are reliable for clinically "actionable" mutations, but that manual curation is required for other mutations. A programme of this scope underscores the need for more standardisation and harmonisation between sequencing platforms. Copy number alteration (CNA) detection by targeted gene sequencing (TGS) is challenging due to false negatives because the density of loci assessed by TGS is low, and false positives caused by background noise arising from fresh frozen paraffin embedded (FFPE) tissue samples. Strategies must be developed to attain the lowest possible false negative and positive rates.

BIG is proceeding with the AURORA initiative and is endeavouring to enrol 1300 patients with metastatic breast cancer from more than 80 centres throughout Europe.

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Affiliation and Disclosure

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Disclosure

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