ONCOLOGY PRO
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2013 IMPAKT Breast Cancer Conference

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Brussels, Belgium

SUMMARY

The 5th IMPAKT (IMProvingcAare and Knowledge through Translational research) Breast Cancer Conference, held 2-4 May 2013 in Brussels, Belgium brought together leading investigators and experts in the field of breast cancer. Organised by the European Society for Medical Oncology (ESMO) and the Breast International Group (BIG), this meeting provided a forum for the most recent translational research findings in breast cancer and gave insights into how these data may alter patient care. A multidisciplinary alliance of breast cancer organisations in Europe that included the European Organisation for the Research and Treatment of Cancer (EORTC), EuropaDonna, European Society of Breast Cancer Specialists (EUSOMA), and St. Galen Oncology Conferences collaborated in creating a unique scientific programme. The scope of this report is to present the scientific highlights of the 5th IMPAKT Conference.

INTRODUCTION

More than 540 participants came from 51 countries world-wide to present and learn about the most up to date technological advances, research findings and emerging clinical care strategies in the field of breast cancer. Importantly, the Conference built a bridge between new data (even at the level of basic research) and clinical practice. Laboratory discoveries were translated and tested in clinical practice environments in clinical trials and hypotheses refined with investigators, allowing them to build upon this knowledge and move forward in their further investigations.

Among the major themes of the Conference were thegenetic heterogeneity of breast cancer including: mechanisms, how to capture it and the corresponding clinical implications, methods to target and further characterisethe PI3K/AKT/mTOR signalling pathway, tumour reprogramming induced by treatment, and new biomarker driven neoadjuvant breast cancer trials.

A specific pre-IMPAKT training course was aimed at providing early-career professionals with the 'must know' fundamentals of research and novel technologies used in the field of breast cancer. The course was attended by 94 young doctors and, for the first time, featured topics relevant for training of pathologists. In addition, there was a section of the program focussed on pathology for non-pathologists, molecular techniques, basic science, and tips for good translational and clinical research.

Figure 1. A detail from the IMPAKT 2013 Training course.

The oral and poster presentations provided information about advances made in breast cancer research on the molecular level and created an understanding for the way in which changes on this level may determine response to treatments used in clinical practice. For the first time, presentations with original research data were incorporated into educational sessions on the relevant target or technique.

The Conference was designed to allow networking and provided opportunities for attendees to meet top experts in the breast cancer field, share information and form new collaborations. Participants left the Conference with not only a high-quality overview of current translational research, but also new inspiration and renewed focus in their endeavour to provide the best medical care available to their

patients with breast cancer.

The program of 2013 IMPAKT Breast Cancer Conference began with reports on breast cancer heterogeneity. Several presentations estimated the impact of cellular heterogeneity within the tumour upon diagnostic and genetic testing.

HETEROGENEITY OF BREAST CANCER: HOW MUCH IS THERE AT THE START AND HOW MUCH HAPPENS OVER TIME?

Intratumoural heterogeneity found to be the primary cause of variance in gene expression microarrays

In order to make genetic analyses more directly applicable to the understanding of tumour response following diverse treatments, RosannaLau, University of Texas MD Anderson Cancer Center, Houston, USA presented data evaluating the factors that influence results from microarray-based measurement of gene expression, or multigene signatures. Total variance was attributed to a composite of a signal representing biological variance plus noise, which represents intratumoural heterogeneity, pre-analytical sample integrity and analytical assay error. Of the noise components, study findings indicated the primary cause of variance between biopsies from the same tumour to be intratumoural heterogeneity. However, the extent of that variation depended on the particular gene or groups of genes being studied.

Intratumoural heterogeneity, or the extent to which the tumour is comprised of different cell types, was estimated from three separate biopsies taken from 51 breast cancers using Affymetrix U133A arrays. While not the major source of variance, technical aspects were found to contribute to overall variation; the study authors suggested this could be minimised by pooling samples from tumour sites. The analytical variance (AV) within the technical procedure was determined by estimating the AV of each key step of the standard microarray profiling procedurein a single biopsy from 20 of the 51 tumours. Cohort data previously published by Hatzis et al. (JNCI 2011) was used to estimate the pre-analytical variance (PAV) from 6 levels of ex vivo ischemia in 11 breast cancers. Total within sample variance for each study was calculated using a linear mixed effects model. Analytical variance was found to be affected very little by the procedures of cDNA synthesis and in vitro transcription, which were highly reproducible. The AV of measurements of single genes (ESRI, MKI67) and multigene indices (SET index and GGI) were most affected by RNA extraction and array hybridization (Figure 2). The standard deviation (SD) associated with MKI67 measurements was approximately 70% of the biological SD, limiting the clinical utility of this marker (Figure 3). For othergenes, notably for ESRI, the SET index and GGI, the total measurement SD accounted for 18-33% of the biological SD and had acceptable reproducibility. The contribution was found to vary according to each gene or

signature but intratumoural heterogeneity dominated other contributions overall.

In general, intratumoural heterogeneity, analytical and pre-analytical variance contributed to approximately 30% of the total standard deviation in single and multigene measurements. Quality improvement in the clinic might include pooling two or more biopsies, optimizing sample preservation and laboratory attention to RNA extraction and array hybridization.No conflicts of interest were disclosed. (Lau, et al. Abstract 40O_PR)

Practice point and future research opportunities

Gene expression measurements from human breast cancers are most affected by intratumoural heterogeneity. Accuracy in multi-gene signatures may be improved by performing them with two or more pooled biopsy samples rather than a single biopsy of the tumour.

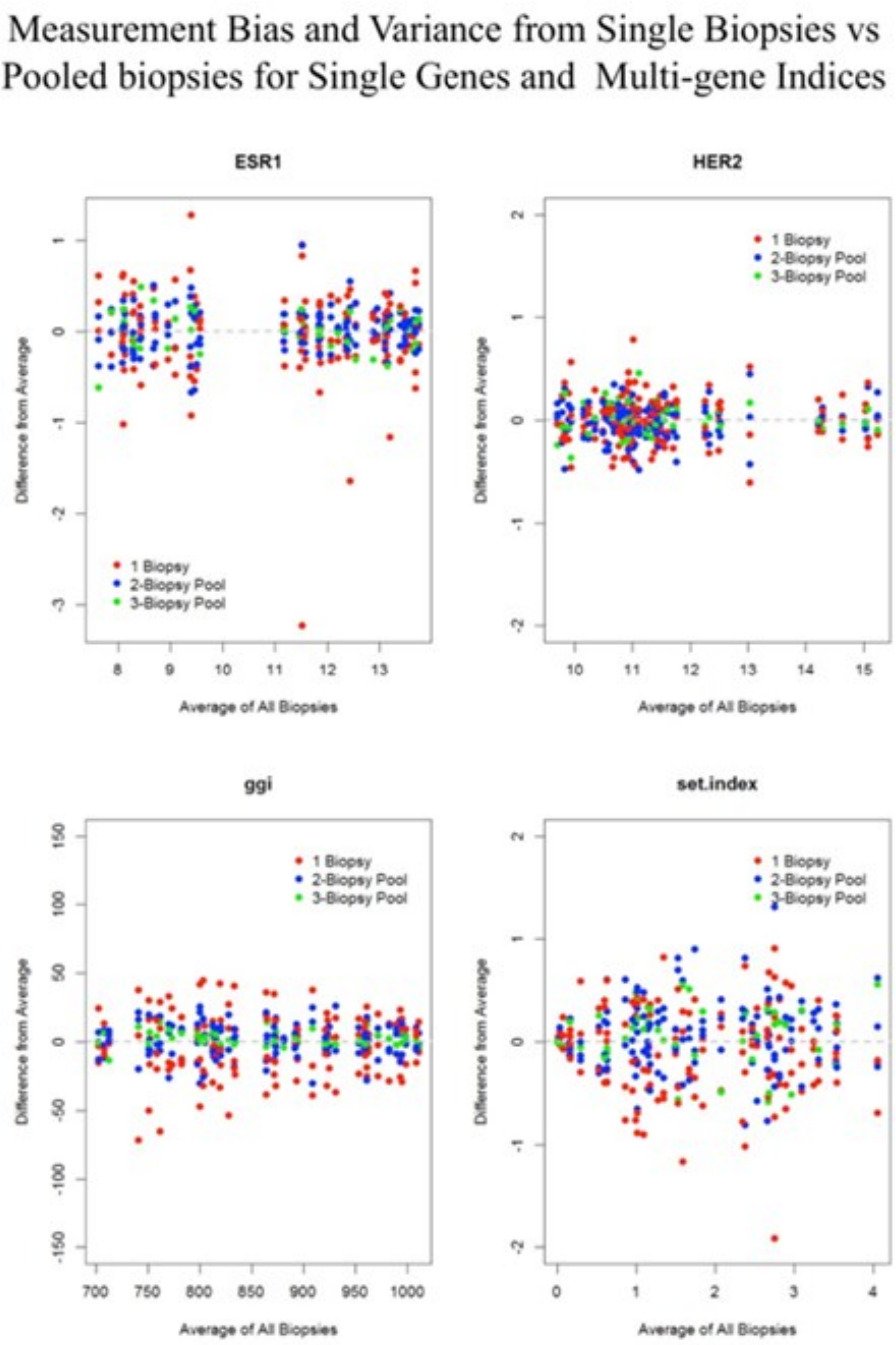

Figure 2. Measurement bias and variance from single biopsies vs pooled biopsies for single genes and multigene indices.

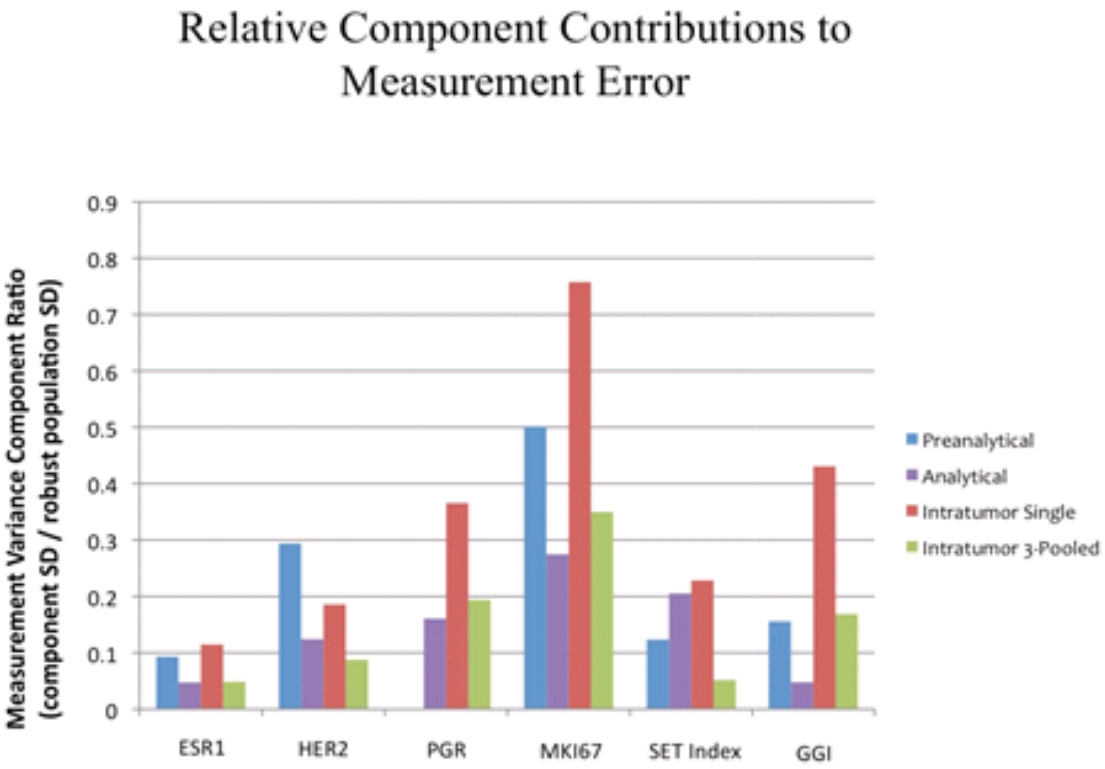

Figure 3. Relative component contributions to measurement error

Tumour heterogeneity contributes to variability in genomic signatures but should not deter predictive testing

During the same session, Micha Jarzb presented findings on behalf of colleagues from the Maria Skodowska-Curie Memorial Cancer Centre and the Institute of Oncology, Gliwice, Poland from their study which was also directed at improving the accuracy of genetic testing in breast cancer tumours. The ability of genomic signatures to define the tumour characteristics can be compromised by working with small specimens like core biopsy samples that often do not contain sufficient percentage of the tumour cells in the specimen. This study tested the strategy of obtaining three independent cores for molecular analysis and assessed the influence of the stability of different prognostic and predictive signatures. Of 302 patients with breast cancer recruited prospectively for the study, 26 were selected during the first year to cover both early and advanced disease; 10 patients had HER2-negative disease, 9 had triple negative and 7 were HER2-positive. All patients received pre-operative chemotherapy. Three cores from different tumour areas in each patient were sampled for molecular testing and were independent of the cores collected for histopathological analysis (Figure 4). Gene expression profiling of tumours was done by using Affymetrix HG-U133 Plus 2.0 microarrays in the subset of 3 independent samples per 26 patients for a total of 78 arrays. After assessing the set of 32 preselected prognostic and predictive signatures, it was concluded that gene expression within the

signatures was significantly better than for random sets of genes. However, differences between the signatures were also observed: The authors advised that at least a 10% coefficient of variability could be expected between replicate samples. Heterogeneity was seen in 5 (25%) patients andwas significant enough to negatively impact the genomic assessment if it had been carried out using just one sample. The authors also found that multigene signatures differed in the variability of the genes comprising the signature; immune response-related genes displayed the most heterogeneity while classifiers based on genes selected from both cell culture experiments and patient tissues were the least heterogeneous.The authors estimated that the 10% variability in each gene could be reduced or augmented by the gene content of the array and algorithm used. The study was funded from the Polish National Science Centre grant N402 6861 40. (Jarzab, et al. Abstract 41O)

Practice point and future research opportunities

The presence of significant heterogeneity within breast tumours was confirmed. The application of three independent cores for genomic analysis in breast cancer may be a successful strategy to overcome tumour heterogeneity and sampling error, and may provide more stable results of prognostic and/or predictive signatures

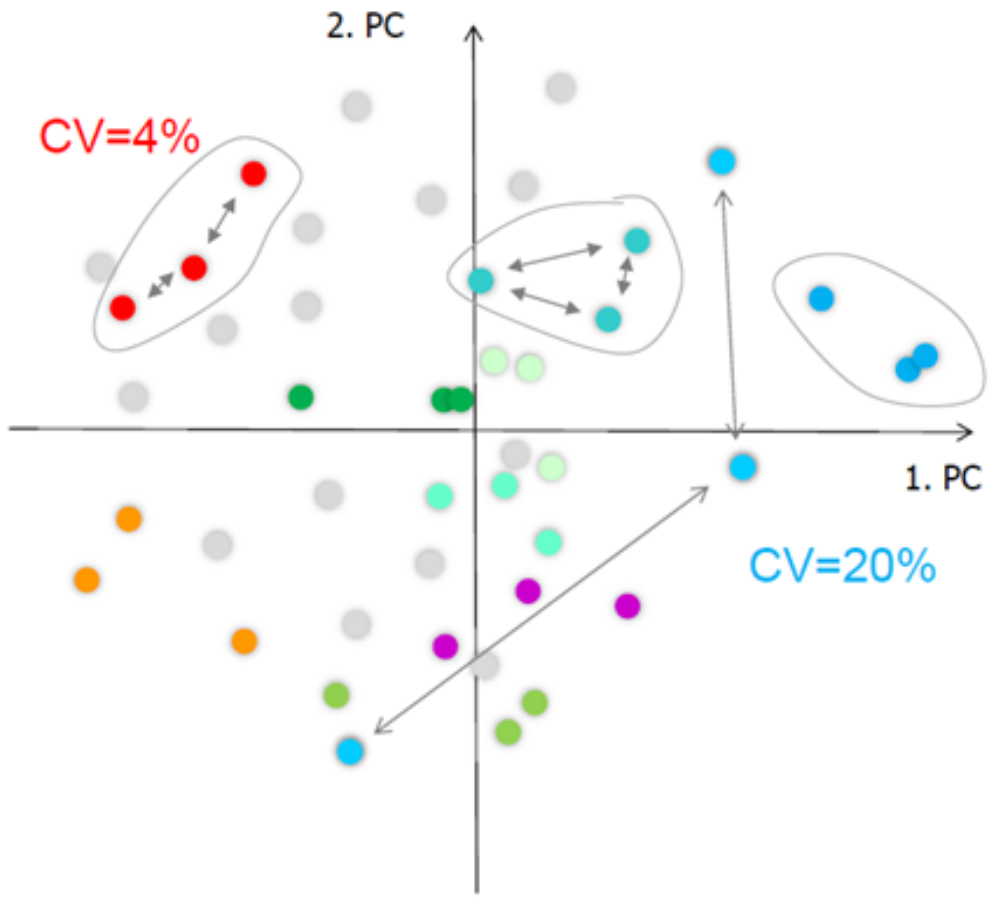

Figure 4. Relatively homogenous pattern of three samples from each patient.

CAN HOST IMMUNE RESPONSE BEAT TUMOUR HETEROGENEITY OR DOES IT COMPLICATE OUTCOMES?

Presence of follicular helper CD4+ T cells in extensively-infiltrated breast tumours signal an organized immune response and predict better outcome

Karen Willard-Gallo from the department of Molecular Immunology of the Institut Jules Bordet in Brussels, Belgium presented findings on behalf of colleagues regarding an immune signature that was predictive of improved long term survival in 794 untreated breast cancer patients who achieved survival of >10-years (p=0.0036).This gene signature was also predictive in 996 patients who received preoperative chemotherapy; of these, 23% of patients achieved pathological complete response.

The signature was identified following the group's characterisation of the T cell infiltrate (TIL) in breast tumours. They profiled the TIL from isolates of 70 untreated invasive primary breast tumours and then compared it with T cells found in non-adjacent breast tissue, lymph nodes and blood. The TIL profiles showed that the concentration of CD4+ cells was higher in tumour than incontrol tissues. Follicular helper T (Tfh) predominated in the TIL and were the primary source of the B cell chemoattractant CXCL13. Other components of TIL were Th1, Th2 and Th17 effector memory and T regulatory subpopulations. Direct comparison of extensively versus minimally-infiltrated tumours led to the finding that extensive immune infiltrates had a larger population of Tfh cells and were organized into tertiary lymphoid structures (TLS), which indicated organized immunity resulting in less suppression and more T cell activation. According to the authors, the presence of TLS is associated with a higher response rate to chemotherapy and/or excellent long-term clinical outcome, and is an important prognostic factor. They reasoned that TLS may play a role in promoting an anti-tumour response, in protecting from tumour mediated immunosuppression, in the generation of long term immunological memory, or perhaps all three. All authors have declared no conflicts of interest. (Gallo, et al. Abstract 80O)

Practice point and future research opportunities

The presence of tertiary lymphoid structures in breast cancer tissue may reflect enhanced immune activity and be an indicator of better clinical outcome.

PI3K/AKT/mTOR: TARGETS/TRIALS AND BIOMARKERS

Next-generation sequencing identifies genetic alterations in postmenopausal women with hormone

receptor positive, HER2-negative advanced breast cancer that alter signalling pathways, tumour suppression and protein binding

During the session on the PI3K/AKT/mTOR pathway, Martine Piccart, Department of Medicine, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium presented findings from a genetic analysis of data from the BOLERO-2 trial. The study enrolled 724 postmenopausal women with hormone receptor positive, HER2-negative advanced breast cancer and demonstrated that everolimus plus exemestane significantly improved progression-free survival, response rate, and clinical benefit rate over placebo plus exemestane. Benefit from the drug combination was observed across all subgroups but variations in response were observed, which the authors reasoned could be partially due to genetic differences in molecular determinants of everolimus sensitivity and interactions between the estrogen receptor (ER) and mTOR pathways.

Dr Piccart contributed to the design of the BOLERO-2 trial and the recruitment of patients; however the BOLERO-2 translational study was carried out by Novartis Pharmaceuticals and Foundation Medicine. They used next-generation sequencing to identifythe genetic alterations in archival specimens from 230 tumours from patients included in the trial. Following DNA extraction, the coding regions of 182 cancer-related genes were analyzed for sequence and copy number variations which showed that all (100%) patients had one or more genetic alterations and 98% of patients had two or more alterations in these regions.The results were consistent with the Cancer Genome Atlas and with previously published results for ER-positive/HER2-negative breast cancer, except that a higher proportion of mutation rates for ARID1A, ESR1 and BRCA2 were found in this study. Somatic variations included 216 missence mutations, 128 nonsense/frameshift/splice/insertion/deletions, 516 amplifications, 26 biallelic deletions, and 3 rearrangements. PIK3CA mutations were most often seen and occurred in 49% of samples, TP53 in 24%, and ARID1A was mutated in 7% of samples. PIK3CA mutations were noted in 112 patients, occurring most often at exons 20 and 9 in 55 and 43 patients, respectively. AKT1 mutations were documented in 6% of samples and were mutually exclusive with PIK3CA mutations. Amplifications involving CCND1 were found in 31% of samples and FGFR1 in 18% of samples. Approximately 66% of samples had one or more amplification event and 8 or more sequence variations without gene amplification were seen in 10% of samples.

Mutations in the estrogen receptor (ESR1) were enriched in metastatic samples (19%) compared with primary tumour samples (7%); a cluster of 11 mutations was seen in the ESR1 ligand binding domain between amino acids 535 and 538 that could affect the affinity and binding kinetics of oestrogen. PTEN mutation associated with low PTEN immunohistochemistry scores; 100% of tumours with PTEN mutations had a score of 0 to 5 and no PTEN mutations were seen in patients with a score more than 5. Results from this study will be further investigated in the large pan-European effort called PRISM and led by the Breast International Group (BIG). This study was funded by Novartis. Dr. Piccart disclosed serving on the board of PharmaMar and acting as a consultant for Sanofi-Aventis,

Amgen, Roche Genentech, Bayer, and AstraZeneca. Other disclosures included grant support and/or honoraria from Sanofi-Aventis, Amgen, Roche Genentech, Bayer, AstraZeneca, Pfizer, Boehringer Ingelheim, Bristol-Meyers Squibb, GlaxoSmithKline and Novartis. (Piccart, et al. Abstract 42O_PR)

Practice point and future research opportunities

BOLERO-2 demonstrates the feasibility of large scale next generation sequencing and shows it can be applied to 'real-life' samples of patients. Large-scale sequencing in phase III trials will potentially provide an understanding of why some patients show a good clinical response to the investigated drugs and others do not. Some mutations were found to cluster into similar pathways, which opens the door to the development of novel targeted therapies for women with hormone receptor positive, HER2-negative advanced breast cancer.

UNICANCER RADHER trial finds no value of AKT/mTORpathwayimmunohistochemical biomarkers for predicting response to preoperative trastuzumab compared with trastuzumab plus everolimus in patients with early breast cancer

Mario Campone, Department of Medical Oncology, ICO Institut de Cancerologie de l'Ouest René Gauducheau, St. Herblain, France and colleagues evaluated whether the expression of selectedAKT/mTOR pathway, proliferation or apoptosis biomarkers could be used as predictive biomarkers for improved sensitivity to trastuzumab following combination treatment with trastuzumab plus everolimus in patients with early breast cancer (Figure 5). Patients often develop resistance to trastuzumab over time and adding everolimus, an inhibitor of the mTORpathway has been demonstrated to restore trastuzumab response in these patients. RADHER trial results were presented demonstrating enhanced clinical response rates (cRR) in 40 patients who received combination treatment compared to 40 patients receiving trastuzumab alone; however, pathological response rates(pRR) were similar between groups. The response was not statistically significant for cRR, which was observed in 35% and 45% of patients, respectively, who received sole and combination trastuzumab treatment (p=0.36); pRR was observed in 43.5% and 47.5% of these patients, respectively (p=0.73) (Figure 6).

An analysis of immunohistochemistry biomarkers expressed in the mTOR pathway, including p4EBP1, Ki67, pAKT, pS6, eIF4E,LKB1 and caspase 3, was then conducted using tumour samples from 66 patients with HER2-positive primary breast cancer confirmed by immunohistochemistry score 3+ or FISH/SISH positive. No predictive value was seen for these biomarkers and either cRR or pRR following combination treatment. Differential expression of biomarkers pre- and post-treatment was unaffected by treatment with the exception of p4EBP1; however this expression did not correlate with response. Authors Campone, Bachelot and Delaloge disclosed grant/research support from Novartis; Authors Bachelot, Merlin and Delaloge disclosed speakerships/consultancy for Novartis and Roche;

Dr.Campone disclosed speakerships/consultancy for Novartis and Servier and Dr.Diéras disclosed speakership/consultancy for Roche. (Campone, et al. Abstract 56O_PR)

Practice point and future research opportunities

Although it is a rather small trial with a limited number of patients, it quite unequivocally shows that the addition of everolimus to trastuzumab seems to leads to increased clinical responses in HER2 overexpressing breast cancer, as compared to trastuzumab alone. None of the studied biomarkers was able to predict which patients would have clinical benefit. It appears that the combination of everolimus and trastuzumab is effective independently of the activation of the PI3K/AKT/mTOR pathway and without any anti-proliferative and pro-apoptotic effect.

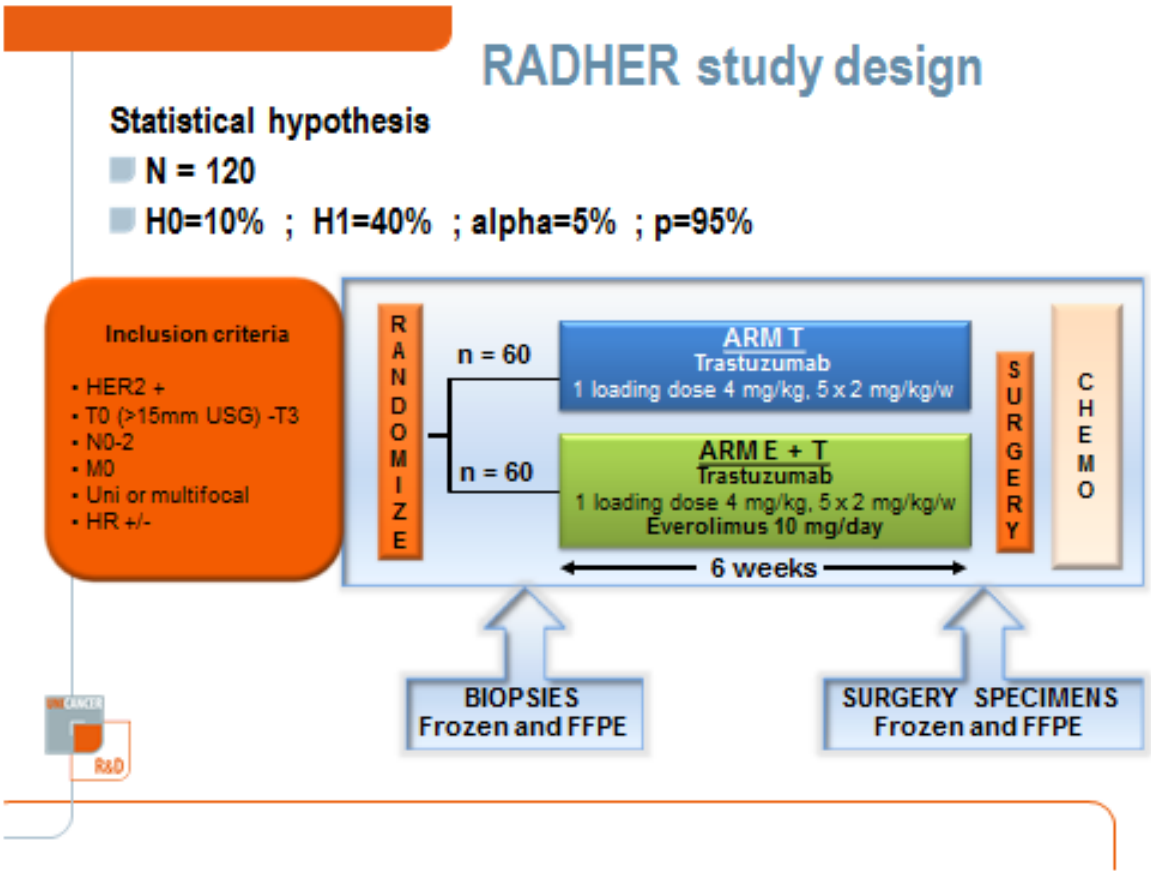

Figure 5. Design of the RADHER study.

	T	E+T	p
Clinical Response Rate			
CR + PR	**35%**	**45%**	0.36
CR	17.5%	25%	
PR	17.5%	20%	
Pathological Response			
pRR (TA+TB)	**43.5%**	**47.5%**	0.73
TA	15.4%	7.5%	
TB	28.2%	40%	

Figure 6. Clinical and pathological response rates in the RADHER study.

Legend: T - trastuzumab, E - erlotinibç

BEST ABSTRACTS SESSION

The best abstract session was comprised of presentations addressing a broad range of topics across the field of breast cancer. Here we present the findings from two groups of abstracts that examined the molecular characteristics of tumours leading to late recurrences and which either identify patients who could benefit from prolonged adjuvant hormonal therapy, or who benefit from a certain adjuvant hormonal therapy which differs from the current practice. The second group of abstracts focuses on studies of imaging techniques for non-invasive measurement at molecular level.

PAM50 risk of recurrence score may be used to identify patients who could benefit from extended adjuvant therapy

Patients with endocrine-responsive breast cancer, even those with initially favourable molecular characteristics such as the luminal A type, have an annual relapse risk that persists well beyond 5 years of follow-up. Since extending adjuvant therapy may be an option to reduce risk of late metastasis, it is necessary to identify patients at high versus low risk of late relapse for clinical decision making regarding treatment extension and for patient counselling.

Michael Gnant, Department Of Surgery and Comprehensive Cancer Centre of the Medical University

of Vienna, Vienna, Austria presented findings on behalf of the Austrian Breast and Colorectal Cancer Study Group from a study that evaluated whether the PAM50 signature could predict late metastasis in a large cohort of postmenopausal women with hormone receptor positive, endocrine responsive breast cancer. Of participants in the ABCSG-8 trial 1,478 patients underwent analysis using a PAM50-based breast cancer gene signature assay. This assay categorises a breast tumour specimen into intrinsic breast cancer subtypes and provides a risk-of-recurrence (ROR) score bydirectly measuring the mRNA expression level of 58 different genes in a single hybridization. The ROR score thus generated has been clinically validated in several studies.

According to the ROR score, 502 (34%), 478 (32%) and 498 (34%) patients were characterised as being at low, moderate and high risk, respectively, for late disease recurrence. These risk groups had significantly different outcomes as to late distant-relapse-free survival (DRFS) (Figure 7) and late relapse-free survival (RFS).At a follow-up of a median 11 years, 98.7% of PAM50 low risk patients experienced no late metastasis between 5 and 10 years of follow-up compared to 91.5% of patients in the high risk group. The score was predictive for patients with node-positive and node negative disease. The PAM50 ROR score was found to significantly relate to the probability of late metastasis. The metastatic risk in PAM50 high risk patients was 8.5% between years 5 and 10 and rose to 9.0% between years 10 to 15, suggesting they may be candidates for extension of adjuvant therapy. By contrast, PAM50 low risk patients may be spared extended adjuvant therapy and concomitant side effects since their risk was determined to be 1.3% between years 5 to 10 and to lower to 1.2% between years 10 to 15. PAM50 ROR together with established clinicopathological risk factors can be used to differentiate patients according to their risk for late metastasis. Dr.Gnant concluded that it is not known whether outcome can definitely be improved by extending adjuvant therapy in patients identified by ROR score as high risk, but it appears logical that this may be the case. Dr.Gnant disclosed institutional support from Sanofi-Aventis, Roche, Pfizer and Novartis plus Honoraria from Amgen, Sandoz, Genomic Health, Bayer, AstraZeneca, GlaxoSmithKline and Nanostring Technologies. (Gnant, et al. Abstract 53O_PR)

Practice point and future research opportunities

The PAM50 ROR score provided significant prognostic information regarding late distant-relapse-free survival and may be used to identify patients with endocrine-responsive breast cancer who are at risk for late metastasis and could therefore possibly benefit from extended adjuvant therapy. Conversely, patients at low risk who can be spared the concomitant cost and side effects of prolonged treatment may also be identified.

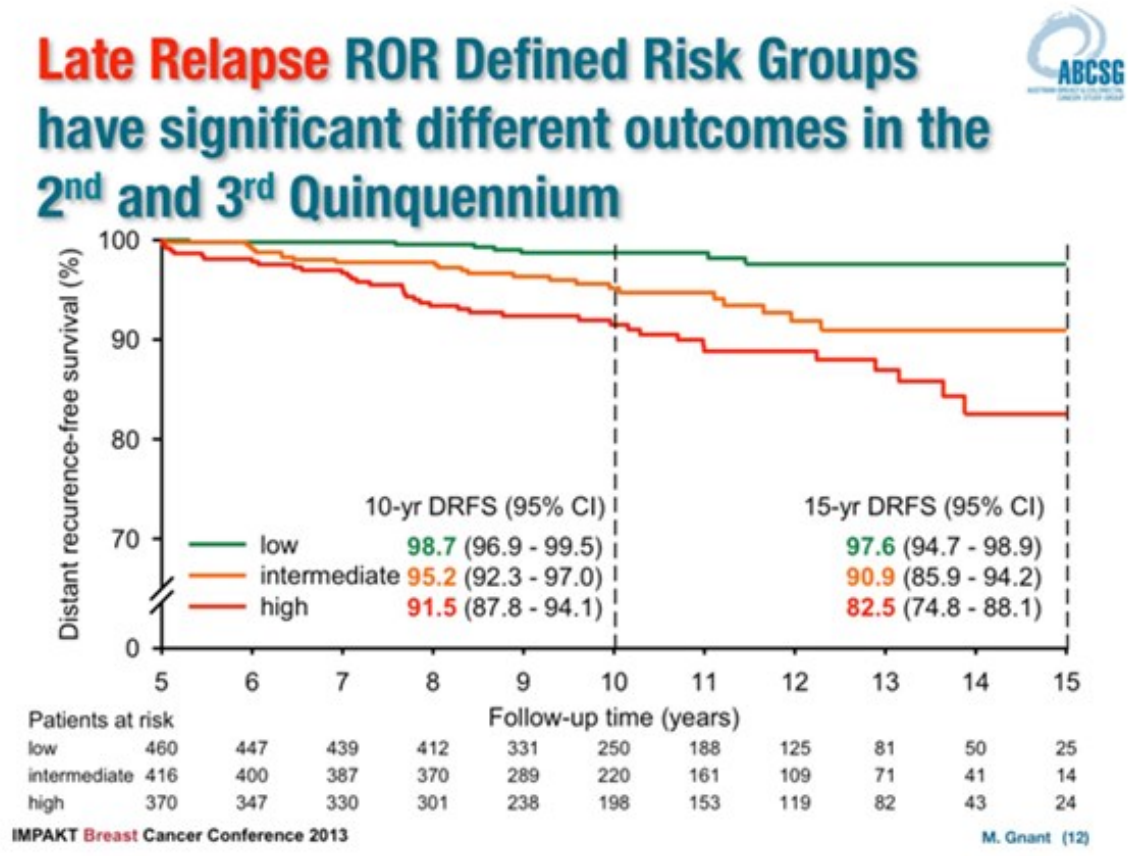

Figure 7. Late relapse ROR defined risk groups have significantly different outcomes upon the 10 and 15 years of follow-up.

Comparison of five different scores pinpoint two molecular scores that may be predictive for late recurrence of oestrogen receptor positive breast cancer in addition to the Clinical Treatment Score

Ivana Sestak, Wolfson Institute of Preventive Medicine, Centre for Cancer Prevention, London, UK, presented results from the transATAC study which compared the accuracy of five different scores in predicting distant late recurrence in patients with oestrogen receptor (ER)-positive breast cancer. For that purpose, data from 891 patients who received either sole tamoxifen or anastrozole in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial, and who were scored using all five tests were evaluated. Patients were followed-up for a median of 10 years.

Dr. Sestak stressed the need for finding prognostic factors for late distant recurrence that could be used in identifying patients with sufficient residual risk to merit administration of adjuvant endocrine therapy beyond the 5 years currently recommended, which has demonstrated benefit in some patients with ER-positive breast cancer. This group showed previously that four immunohistochemical (IHC) markers (ER, PgR, Ki67, HER2), plus specific clinical variables were significantly associated with time to distant recurrence. The current study compared the value of the Clinical Treatment Score (CTS), IHC4 score, the OncotypeDx Recurrence Score (RS), the PAM 50

Risk of Recurrence (ROR) and the Breast Cancer Index (BCI) score in predicting risk of distant recurrence, in years 0 to 5 and 5 to 10 post diagnosis. Similar results were seen for the node-negative and node positive populations. All scores provided information regarding distant recurrence during the 0 to 5 year period; however, univariate analysis favoured the CTS as adding the most prognostic information years in 0 to 5 (Figure 8).Longer term prognostic information was given by CTS, followed by ROR and BCI which both added significant prognostic information for distant recurrence beyond the CTC. The CTS is made up of tumour size, grade, nodal status, patients age and treatment received, ROR contains tumour size and a 50 gene panel while the BCI is composed of HOXB13/IL17BR and a Molecular Grade Index of a 5 tumour grade gene signature. CTS was identified as the strongest prognosticator for distant recurrence in both time periods, primarily driven by nodal status and tumour size, with ROR and BCI being the only molecular scores that added substantial prognostic information during years 5 to 10 (Figure 9). These data suggest that ROR and BCI may be used to identify patients at risk for late distant metastases. The group now plans to investigate which individual genes and components that comprise these scores contribute most to the prediction of late recurrence and may allow the identification of patients at high risk of recurrence that may benefit from treatment extension. Drs.Dowsett and Cuzick disclosed grant support from and membership of the speakers bureau of AstraZeneca; Dr.Dowsett also is an advisor to Genoptix. Dr. Erlander is an employee of and owns stock in Theranostic, INC. Dr.Cowens and Feree are employees and shareholders of NanoString Technologies. Drs. Sestak and Sgroi had no disclosures. (Sestak, et al. Abstract 54O_PR)

Practice point and future research opportunities

This study identifies the Clinical Treatment Score as being the strongest predictor of distant late recurrence following treatment in patients with oestrogen receptor positive breast cancer. The Breast Cancer Index score and the PAM50 Risk of Recurrence Score, which both contain genetic information not included in the Clinical Treatment Score that is not routinely measured in clinics, also provided information for late recurrence. Further validation is needed before incorporating the ROR and BCI into clinical decision making.

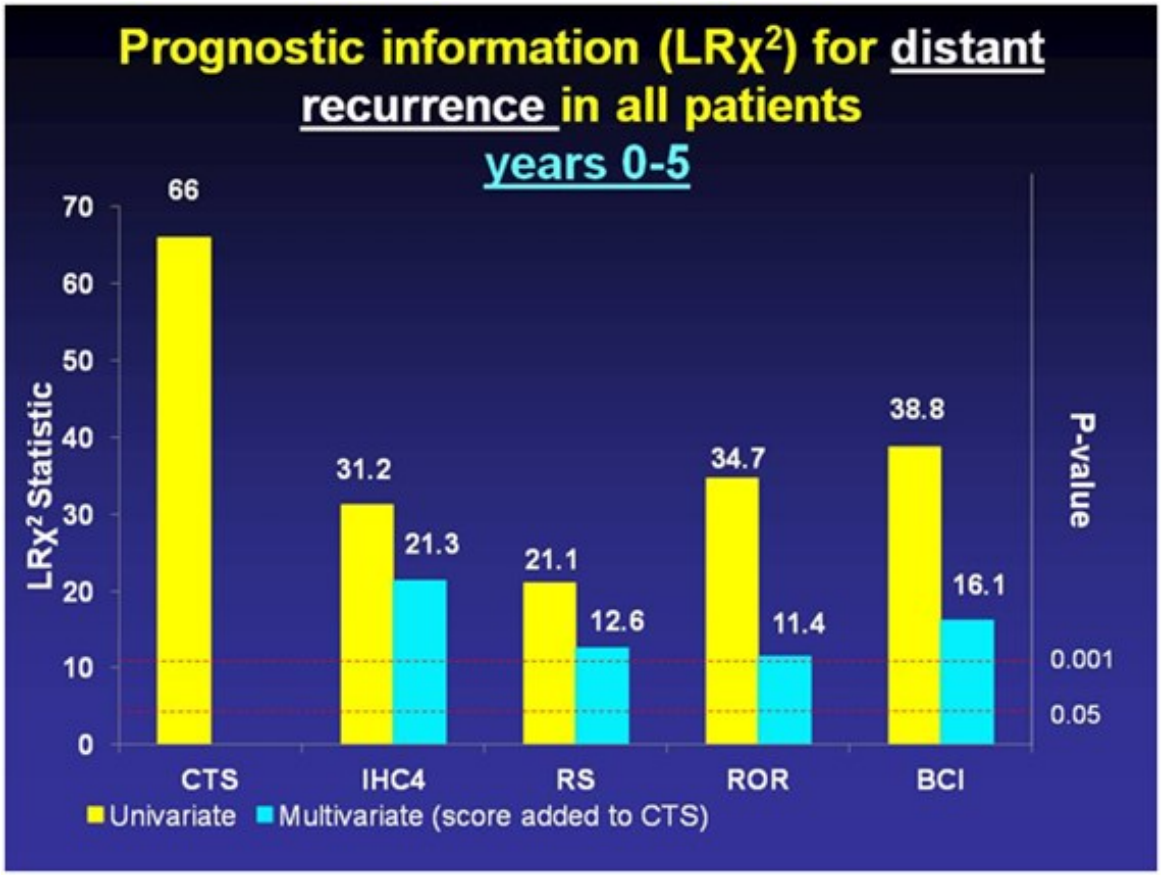

Figure 8. Prognostic information for distant recurrence in all patients from 0 to 5 years.

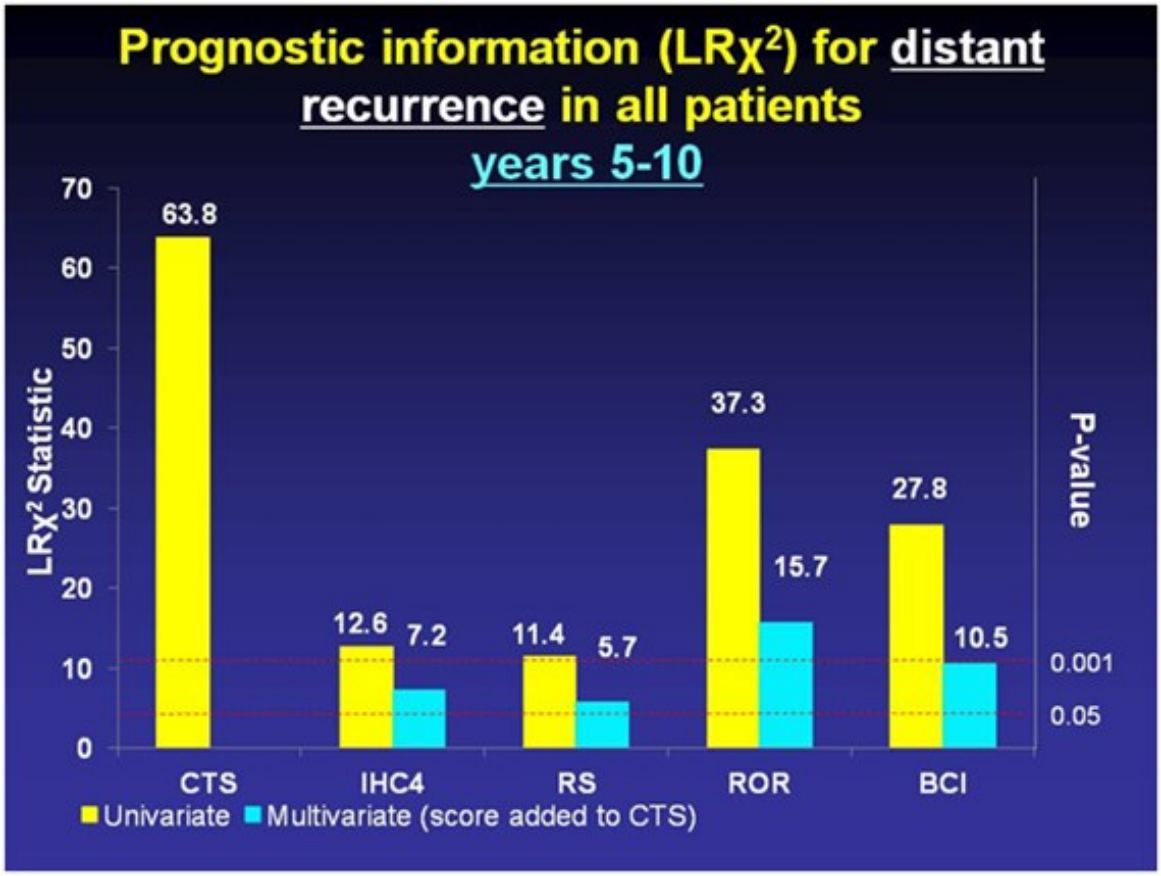

Figure 9. Prognostic information for distant recurrence in all patients from 5 to 10 years.

High ERß1 expression, PR positivity and low Ki67 expression favour improved outcome with continued tamoxifen treatment of postmenopausal women with ER-positive early breast cancer

Giuseppe Viale, Department of Pathology, Istituto Europeo di Oncologia, University of Milan, Milan, Italy reported findings on behalf of the PathIES investigators from the retrospective Path Intergroup Exemestane Study (PathIES) study that evaluated whether high expression of tumour markers was associated with improved outcome and could be used to identify postmenopausal women with ER-positive early breast cancer who could benefit from continuing tamoxifen treatment or switching to exemestane. Tissue samples from 1,256 (27%) participants in the Exemestane Study Group trial were studied; the samples were taken at least two years prior to the study, which evaluated data from 2,372 women who received sequential exemestane and 2,353 women receiving further tamoxifen following 2 to 3 years of tamoxifen treatment for early breast cancer. Biomarker expression of ER, PR, HER2, Ki67 and ER1 was determined by immunohistochemistry (IHC4) and evaluated as predictors of patient outcome; the prognostic value of IHC4 was tested by the time-to-distant recurrence.

In both treatment groups, high IHC4 scores were associated with worse prognosis. Tertile comparison of tissue IHC4 scores for time to distant recurrence confirmed the prognostic value of IHC4 across hazard ratios (HRs): second tertile versus the first tertile, 1.45; and third tertile versus first tertile, 2.32 (p=0 .04). Combining IHC4 with a clinical score comprised of node status, grade and tumour size increased the strength of these results: second tertile versus first tertile, 3.7; and third tertile versus first tertile, 9.1 (p<0.001). Multivariate analysis revealed no significant association between ER1 expression and either disease-free survival (DFS) or overall survival (OS) with exemestane treatment (HR 1.11); however, high ER1 expression significantly correlated with improved DFS in patients treated with tamoxifen (HR0.38; p=0.01). Interactions were also seen for the continuous expression of ER1/PR and improved DFS (HR 0.966; p=0.03) and for PR positivity and DFS (HR 0.28; p=0.03) under tamoxifen treatment. Conversely, high Ki67 expression was significantly associated with worse DFS (HR 1.70). Dr. Viale concluded that, although the ERß1 results were interesting, they require independent validation as this was the first time it was examined in the context of a trial. Authors Bliss and Coombs disclosed research grants from Pfizer; no other potential conflicts were disclosed. (Viale, et al. Abstract 55O_PR)

Practice point and future research opportunities

High ERß1 expression, PR positivity and low Ki67 was associated with improved disease-free survival and overall survival for tamoxifen-treated patients and suggests that these patients should

receive 5 years of adjuvant treatment with tamoxifen rather than switching to exemestane. The utility of the IHC4 score with and without clinical variables for prognostic information in these patients was confirmed.

Live in vivo lymphatic imaging techniques show immune cell interactions in a syngeneic mouse model of breast cancer

Sheeba Irshad of the Research Oncology department of the King's College London School of Medicine, London, UK raised the issue of "cancer immunoediting" which describes the interaction of the immune system's host-protection response with tumour promotion and attempted to characterise the abnormal cell interactions that favour tumour development. Her team used breast cancer animal models to visualize immune cell trafficking and interactions within the lymphatic tissue. Mammary carcinoma cells 4T1 and the daughter clone 4T1.2 were injected into the mammary fat pads of syngeneic female Balb/C mice. Immunohistochemistry and flow cytometry were used to define tumour load and changes in the immune cell populations within lymph nodes (LN) and spleens. A mammary imaging window, as described in previous studies, was also used, to describe tumour development. Interactions between immune cells isolated from BALB/c mice, which had been fluorescently labelled ex-vivo and re-injected into tumour-bearing mice were visualized within the primary tumour at multiple timepoints by multiphoton microscopy.

Study findings were consistent with previous reports; positive staining for Pan-CK and E-cadherin in LN sections indicated that 4T1.2 tumours metastasise preferentially via the lymphatics compared to 4T1 tumours, which metastasise via the haematogenous route. Analysis of LNs from 4T1.2-tumour bearing mice at early stages of tumour development showed increased numbers of B cells with a phenotype similar to that described for regulatory B cells (Bregs). Accompanying the increase in this B cell subset was an increase in CD4 + Tregulatory cells. The mammary imaging window disclosed growth of new lymphatic vasculature in 4T1.2 tumours in vivo at multiple time-points. New lymphatic branches along the main lymphatic vessel efferent to the inguinal LN containing metastasis were observed in 4T1.2 tumour bearing mice. Metastatic 4T1.2 tumour cells where shown interacting with Bregs by multiphoton microscopy. Data from this model suggest that growth of new lymphatic vasculature facilitates tumour metastasis and the microenvironment created by the presence of immune cells may be necessary for lymphangiogenesis. All authors have declared no conflicts of interest. (Irshad, et al. Abstract 89O)

Practice point and future research opportunities

In vivo imaging provides an important tool for defining the immune component of lymphatic metastases of breast cancer and showed B and T regulatory cell populations to be increased during early stages of tumour development. New lymphatic vasculature may facilitate tumour metastasis and

the microenvironment created by the presence of immune cells is necessary for lymphangiogenesis in this model.

Novel 18F-tagged antibody against HER2 may allow non-invasive assessment of HER2 status in vivo by PET

Duncan Hiscock of Medical Diagnostics, R&D, GE Healthcare, Amersham, UK presented findings from a study that aimed to evaluate the HER2-targeting Affibody molecule GE226 ([18F]FBA-ZHER2:2891) in a preclinical tumour model asan agent for non-invasive in vivo imaging of HER-2 status. The determination of HER2 status is crucial in the clinical management of breast cancer patients since it allows for selection of patients who are candidates for HER2 targeting therapies. This study evaluated the pharmacokinetics, specificity and selectivity of the Affibody by positron emission tomography (PET) in a mouse model that has a tumour with high HER2 status (NCI-N87) and a low HER2 status tumour (A431) xenograft in separate flanks. Either the 18F-labelled ZHER2:2891 Affibody molecule GE226 or its fluorescein-labelled analogue was studied in vivo by PET following injection of 4 mice with GE226. Imaging was done at 30, 60 and 120 minutes post injection and the antibody levels in each tumour were quantified using a c-erbB2/c-neu ELISA.

Antibody uptake corresponded well to the known HER2 expression patterns of each tumour; at 120 minutes post-injection these methods showed increased levels of antibody uptake in the tumour, tumour/blood ratios and tumour/muscle ratios that were 2 to 6 times higher in NCI-N87 high HER2 status tumours compared to A431 tumours in each individual mouse. Uptake of GE226 antibody in NCI-N87 tumours increased from 30 to 60 minutes post injection and stayed constant until 120 minutes. Uptake in the low expressing A431 tumour continuously decreased over time upon injection. The GE226 uptake in pooled tumours showed good agreement (Pearson r = 0.84) with the amount of HER2 expressed on these tumours.

In vivo localisation of the Affibody molecule in the NCI-N87 tumours was demonstrated by ex-vivo microscopy of tumour sections taken from mice injected with a mixture of fluorescein-labelled ZHER2:2891 and Hoechst 33342. Further evidence of specificity was demonstrated by the co-localisation of Fluorescein-labelled ZHER2:2891 with HER2 identifiedby the DAKO HercepTest in NCI-N87 tumours. All authors are employees of MDx Discovery, GE Healthcare, which provided funding for this study. (Hoppmann, et al. Abstract 90O)

Practice point and future research opportunities

The Affibody molecule GE226, a HER2 imaging agent, binds HER2 in vivo and uptake correlates with HER2 expression levels in preclinical models of breast cancer using PET technology. A clinical study is planned to detect HER2 expression in patients with breast cancer.

An in vitro model using HER2-positive, trastuzumab resistant breast cancer cells affects 18F-FDG uptake following PI3K-AKT-mTOR pathway blockade

Aberrant activation of the PI3K-AKT-mTOR pathwayis an important driver of resistance to trastuzumaband other HER2 targeted therapies.This pathway is also involved in glucose homeostasis, making fluorodeoxyglucoseemission tomography (18F-FDG-PET), which employs a radiolabeled glucose molecule as a tracer, a candidate for assessing early response to therapies targeting the PI3K-AKT-mTOR pathway. Since information on the effects of blocking this pathway on the uptake of 18F-FDG-PET is scant, Yanina Dockx of the Molecular Imaging Centre Antwerp, University of Antwerp in Edegem, Belgium and colleagues investigated this issue using a HER2-positive breast cancer cell-line, the JIMT-1, which is both resistant to trastuzumab and sensitive to everolimus. Following administration of trastuzumab and dual pharmacological blockade of this pathway was attained with everolimus, an mTOR inhibitor and PIK90, which inhibits PI3K, the inhibitory concentration (IC50) at 72 hours post treatment of all agents was determined by xCelligence and used as reference dose. Thereafter, the cells were treated for 24, 72 and 96 hours with all agents at the determined dose and incubated with FDG. FDG uptake was measured in the cell suspensions and supernatant media with appropriate corrections for radioactive decay and for the number of viable cells. IC50 concentrations of everolimus and PIK90 at 72 hours were 9.5 nM and 35 µM, respectively but the IC50 of trastuzumab was not reached. Everolimus increased FDG uptake by 130% and 167% at 24 and 72 hours ($p<0.05$), respectively, but decreased FDG uptake by 77% at 96 hours. PIK90 treatment decreased FDG uptake at all time points. FDG uptake with trastuzumab was consistently higher compared to control and the other agents. All authors have declared no conflicts of interest. (Dockx, et al. Abstract 91O)

Practice point and future research opportunities

Blockade of PI3K-Akt-mTOR in trastuzumab resistant HER2-positive breast cancer affects in vitro 18F-FDG uptake in transient and opposite ways, depending on the pharmacological target and duration of treatment. Therefore, further validation is necessary to elucidate the cellular mechanisms involved in tracer uptake prior to routine clinical use for early response assessment.

RELATED INFORMATION

Click here to access the Conference abstracts.

Click here to access the meeting webcast page.

Save the date: IMPAKT Breast Cancer Conference 8-10 May 2014, with the pre-conference training

course, 7-8 May 2014.

AFFILIATION AND DISCLOSURE

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Disclosure

Professor Tutt has declared no conflict of interest.

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