Pathways and biomarkers in breast cancer: latest evidence

Gordon Mallarkey and R. Charles Coombes

Ther Adv Med Oncol

[2012] 4(4) 157–166

DOI: 10.1177/
1758834012450600

© The Author(s), 2012.
Reprints and permissions:
[http://www.sagepub.co.uk/
journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

The IMPAKT (Improving Care and Knowledge through Translational Research) breast cancer (BC) conference in Brussels, Belgium (3–5 May 2012) covered the latest developments in translational research.

Martine Piccart (conference cofounder, Institut Jules Bordet, Brussels, Belgium) described how the clonal evolution of tumour progression probably occurs by branching rather than in a linear manner and that some evidence supports parallel progression of primary and metastatic tumours associated with early spread of cancer cells. She said our understanding of heterogeneity between primary and metastatic lesions and among metastatic lesions will dramatically expand in the next few years. The key is to identify the driver mutations. So far, none of the recent translational research studies have identified biomarkers that may be introduced into clinical practice. It is not only the tumour that matters, it is important to investigate the role of the tumour microenvironment. Translational research in advanced disease has added layers of complexity compared with early disease. Dr Piccart believes that progress will only happen provided there is a cultural revolution in the way we conduct such research, and that must include increased collaboration and increased data sharing. This editorial features highlights of the ‘Best Abstracts’ sessions held at the conference.

Biomarkers

Mucin-1 as a predictive marker in clinical immunotherapy trials

The latest results presented by Dr Bruno Sinn (Institut Für Pathologie, Charité Universitätsmedizin Berlin, Berlin, Germany) suggest that mucin-1 (MUC1) expression could be used as a predictive marker in clinical immunotherapy trials [Sinn *et al.* 2012]. MUC1 is expressed in

normal breast epithelium and in invasive BC, and it has pleiotropic effects on tumour biology. Sinn and colleagues evaluated the frequency of MUC1 expression and its predictive value for response and survival after neoadjuvant anthracycline/taxane-based chemotherapy. Using pretreatment core biopsies from patients in the GeparTrio study [von Minckwitz *et al.* 2008] they investigated MUC1 protein ($n = 691$) and mRNA ($n = 268$) expression by immunohistochemistry (IHC) and quantitative reverse transcriptase polymerase chain reaction from formalin-fixed paraffin-embedded samples.

MUC1 protein expression was detectable in 656 (95%) cases and mRNA levels covered 103 copy numbers. There was significant correlation for MUC1 protein and mRNA expression ($p < 0.001$). High MUC1 protein and mRNA expression were seen most often in hormone receptor (HR) positive tumours ($p < 0.001$); lower MUC1 protein and mRNA expression were seen in HR negative (HR–) and human epidermal growth factor receptor 2 negative (HER2–) tumours ($p < 0.001$) compared with other subtypes; low MUC1 protein and mRNA expression were predictive for pathological complete response (pCR) in the overall population ($p < 0.001$) and in HR+ ($p = 0.004$ and < 0.001), HER2– ($p < 0.001$) and HR+/HER2– ($p < 0.001$) subgroups. Multivariate analysis showed that MUC1 protein and mRNA expression were independently predictive.

MUC1 protein expression was prognostic for survival in the overall population ($p = 0.03$) and in HER2– tumours ($p = 0.005$). MUC1 mRNA was prognostic in the overall population ($p < 0.001$), in HR+ ($p < 0.001$), HR– ($p = 0.001$), HER2– ($p < 0.001$) and HR+/HER2– tumours ($p = 0.001$). Multivariate analysis showed protein and mRNA expression to be independently prognostic ($p = 0.029$ and 0.005).

Correspondence to:
**Gordon Mallarkey, PhD,
MRPharmS**
Sage Publications,
1 Oliver's Yard, 55 City
Road, London EC1Y 1SP,
London, UK
mallarkey@sagepub.co.uk
Prof. R. Charles Coombes
Division of Cancer,
Imperial College,
Hammersmith Hospital
Campus, London, UK

The authors concluded that MUC1 expression occurs in a large proportion of patients with BC – especially those with HR+ tumours, and that their evaluation methodology may be useful in providing information on therapy response and survival following neoadjuvant chemotherapy. They also suggest that MUC1 expression may serve as a predictive marker in clinical immunotherapy trials.

Pathways involved in pathological complete response to chemotherapy seems to differ according to different breast cancer subtypes

Michail Ignatiadis (Department of Medical Oncology, Breast Cancer Translational Research Laboratory, Institut Jules Bordet) and colleagues have conducted a study looking at possible associations between pCR and gene modules describing biologically relevant processes and ‘druggable’ oncogenic pathways in BC subtypes [Ignatiadis *et al.* 2012].

They amassed publicly available gene expression data from eight studies consisting of 996 patients treated with anthracycline with or without taxane-based neoadjuvant chemotherapy. From 17 computed gene modules they measured odds ratios (ORs) for pCR for one-unit increases in scaled modules with and without adjustment for clinicopathological characteristics. Subtype analyses were performed [oestrogen receptor (ER) and HER2 status or the PAM50 classifier].

They found higher pCR rates among patients with subtype ER–/HER2– (30%) and in those with HER2+ (36%) compared with ER+/HER2– (11%). An increased pCR was associated with high expression of immune modules across all three subtypes and high expression of chromosomal instability, phosphatase and tensin homolog (PTEN) loss and E2F3 modules in ER–/HER2– and ER+/HER2– but not HER2+ subtype. Insulin-like growth factor 1 (IGF1) module was associated with an increased pCR in ER+/HER2– subtype. Similar results were observed in subtypes based on PAM50. After adjusting for clinicopathological characteristics, high expression of immune module was associated with significantly increased pCR in subtypes HER2+, ER–/HER2–, but no association was found among patients with the ER+/HER2– subtype. High expression of chromosomal instability, PTEN loss, and E2F3 modules were

independently associated with increased pCR in ER–/HER2– and ER+/HER2– subtypes.

The results suggest that the pathways contributing towards a pCR to chemotherapy differ according to different subtypes. The high expression of immune modules seems to be independently associated with an increased pCR in HER2+ and ER–/HER2– subtypes and the authors suggest that novel immune strategies should be tested in these subtypes.

Progesterone receptor-positive tumour cells in immunohistochemistry-defined luminal A breast cancer are of prognostic significance

IHC-based definitions of luminal A (LumA) and B (LumB) BC subtypes are imperfect compared with multigene subtyping assays. Aleix Prat (University of North Carolina, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA) and colleagues have tried to address this issue by looking at the clinical-pathological characteristics of genomically defined LumA and B subtypes and then selecting IHC markers to represent these genomic classes [Prat *et al.* 2012]. They used the PAM50 microarray training dataset to find differences in global and single gene expression patterns between LumA and B tumours, and also collected clinical-pathological data from 2950 primary tumours across four independent studies.

Gene expression analyses identified *PGR* but not *ESR1* as a particularly discriminating gene for LumA tumours. Clinical-pathological investigation found LumA tumours to be associated with higher rates of progesterone receptor (PR) positive tumours, HER2 negativity and histological grade 1 than LumB tumours, but no differences were observed in IHC scoring or ER status of ER+ tumour cells. IHC-defined LumA tumours with a low percentage of PR+ tumour cells were more likely to be identified as non-LumA by PAM50 and were independently associated with lower distant relapse-free survival (DRFS) compared with LumA tumours with a high percentage of PR+ tumour cells. The researchers chose a score of more than 20% PR+ tumour cells to predict survival differences within IHC-defined LumA tumours. In conclusion, IHC expression of PR appears to add prognostic value within the current IHC-based LumA definition by improving the identification of good outcome BCs.

Their definition of LumA would now be HR+/HER2-/Ki67 < 14% and PR > 20%.

HER2 extracellular domain levels are not a predictive biomarker in patients with early breast cancer

HER2 extracellular domain (ECD) levels are elevated in some patients with HER2+ BC and have been proposed as a potential biomarker of therapy response or an indicator for disease progression: ECD elevations greater than 20% from baseline have been associated with disease progression.

Mitch Dowsett (Academic Department of Biochemistry, Royal Marsden Hospital, London, UK) and colleagues performed a nested case-control study in 992 patients with HER2+ early BC from the Herceptin Adjuvant (HERA) trial [Dowsett *et al.* 2012]. Samples were taken at baseline and throughout the study across all three trial arms in a manner to form case-control triplets.

There were 160 case-control triplets across the study: median ECD levels in case samples was 2.4% higher than in control samples. Conditional logistic regression indicated that samples with higher ECD levels were more likely to be a case than a control sample ($p < 0.001$); however, the difference in mean ECD levels between cases and controls was greater than 20% in only 44 of case-control triplets and further statistical analysis did not indicate that the difference in mean ECD levels between cases and controls was greater than 20%. These data were therefore insufficient to support the ECD levels as a predictive biomarker in patients with early BC.

Everolimus more effective in patients with metastatic breast cancer with PI3K-independent activation of mTOR

The GINECO (Group d'Investigateurs Nationaux pour l'Etude des Cancers Ovariens) TAMRAD phase II study included 111 women with HR+ metastatic BC refractory to aromatase inhibitors (AIs) [Bachelot *et al.* 2010]. Adding everolimus to tamoxifen therapy appeared to delay disease progression compared with tamoxifen alone: median time to progression (TTP) 8.6 months *versus* 4.5 months [hazard ratio (HR) 0.53; 95% confidence interval (CI) 0.35–0.81], nonprogression rates at 6 months of 61% *versus* 42%, respectively.

Thomas Bachelot (Medical Oncology, Centre Léon Bérard, Lyon, France) and colleagues sought to determine if a specific patient subpopulation may benefit from adding everolimus to hormone therapy [Bachelot *et al.* 2012]. They collected 51 tumour blocks from the initial tumours of patients in the GINECO study. Hotspot mutation of phosphoinositide 3 kinase (PI3K) (exon 9–20), and KRAS (exon 2) were described, a tissue microarray analysis evaluated IHC expression of mTOR (mammalian target of rapamycin) pathway markers. An exploratory analysis of everolimus efficacy within each biomarker subgroup was conducted (high *versus* low expression defined by median percentage of marked cells). Of the 48 patients assessed for PI3K exon 9 and 20 mutational status and KRAS mutational status, seven had at least one mutation (PI3K exon 9: one patient, exon 20: two patients; KRAS: four patients), which was lower than expected. IHC analysis ($n = 44$) showed a trend for better treatment efficacy in patients with high pS6K, high p4EBP1, low 4EBP and low PI3K. The results indicate that everolimus is more effective in patients with PI3K-independent activation of mTOR. The authors suggest that, if confirmed, this could have important implications for patient selection in metastatic BC.

Identification of prognostic biomarkers in patients with HER2+ breast cancer given adjuvant trastuzumab

There are currently no biomarkers that can identify prognostic groups among patients with BC who have received adjuvant trastuzumab. Debora Fumagalli (Breast Cancer Translational Research Laboratory, Institut Jules Bordet) and coworkers have sought to find prognostic groups in HER2+ BC from a series of such patients in two Belgian hospitals [Fumagalli *et al.* 2012]. The patients had received anthracycline/taxane-based adjuvant chemotherapy plus trastuzumab for 1 year. Frozen tumour samples were available and exposed to hotspot somatic mutation profiling, gene expression profiling and quantification of tumour lymphocytic infiltrate. Regression models were applied to look at associations with relapse-free survival (RFS).

There were 107 women included in the study: median follow up 40.9 months (range 11.2–107 months), 67 were ER+, 59 node positive, 32 T1, 14 relapsed. The researchers evaluated 175 mutations in 22 genes: there were 21 cases of

PIK3CA mutations, two of ERBB2 mutations, six of P53. No mutations were found for epidermal growth factor receptor (EGFR), KRAS, BRAF, NRAS, PI3K, PI3KR1 and PTEN. No significant association of PIK3CA mutations was found for any clinical-pathological factors. Eleven tumours with at least 50% lymphocyte infiltrate [lymphocyte predominant BC phenotype (LPBC)] were associated with excellent survival with no relapses. PIK3CA mutations were associated with a poorer RFS than PIK3CA wild type. Also, in PIK3CAWT tumours, ER+ disease had a significantly better outcome than ER- disease. The authors concluded that LPBC phenotype, PIK3CA genotype and ER status in PIK3CA wild-type tumours can distinguish distinct prognostic groups among patients with HER2+ BC given adjuvant trastuzumab and they suggest further validation in larger datasets is warranted.

Bcl2-negative primary breast tumours but not axillary lymph nodes predict a better survival in patients treated with high-dose epirubicin

Ivana Bozovic (Medical Oncology, Institut Jules Bordet) and coworkers examined the prognostic/predictive role of bcl2 and p53 in primary tumour and axillary lymph nodes in women with node-positive BC treated with adjuvant CMF (cyclophosphamide, methotrexate, fluorouracil) or two different doses of epirubicin-containing chemotherapy [Bozovic *et al.* 2012].

They used IHC in formalin-fixed paraffin-embedded sections of both sites to assess bcl2 and p53 expression in 777 patients assigned randomly to CMF, epirubicin and cyclophosphamide (EC) or high-dose EC. The markers were analysed with regard to overall survival (OS) and event-free survival.

Tumour markers were assessed in 491 cases with a median follow up of 15.6 years. The main findings were as follows: bcl2 positivity in either primary tumours or lymph nodes was associated with better OS; p53 positivity in primary tumours or lymph nodes was associated with a poor outcome independent of type of chemotherapy. In contrast, bcl2- primary tumours were associated with benefit for high-dose EC treatment compared with CMF. Bcl2+ primary tumours predicted an increased benefit with CMF over EC treatments.

PTPN9 may be a biomarker candidate to predict response to HER-targeted therapy

Results from several recent studies suggest that reactivation of mainly HER3 could play an important role in the development of resistance to tyrosine kinase inhibitors and antibodies. The protein tyrosine phosphatase, PTPN9, inhibits EGFR and HER2 but its effect on HER3 is not clear. Merel Gijsen (Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK) and colleagues found that PTPN9 reduces phosphorylation of HER3 and may therefore be implicated in the resistance to targeted therapy [Gijsen *et al.* 2012]. Using an Akt inhibitor they found that the decrease in HER3 phosphorylation was accompanied by an increase in PTPN9. When they knocked out PTPN9 using siRNAs, this blocked HER3 dephosphorylation after Akt inhibitor treatment, which further supported the role of PTPN9 in HER3 dephosphorylation. Their further studies have shown that loss of PTPN9 leads to increased resistance of gefitinib and trastuzumab in HER2+ BC cells. They have investigated the correlation between levels of PTPN9 and survival in tumour microarrays with samples from HER2-overexpressing low levels of PTPN9 correlate with a poor OS and disease-free survival (DFS) in these patients.

Based on these data, the researchers suggest that PTPN9 may be a biomarker candidate to predict response to HER-targeted therapy and that further study in a larger sample population is warranted. They also suggest that increasing PTPN9 by drug therapy may be a new area for drug discovery.

Breast cancer in young women: biologically unique and requiring a distinct therapeutic approach

BC in young women has a poor prognosis and it is not clear if age impacts cancer biology or acts as a surrogate for aggressive BC subtypes. Hatem Azim Jr (Breast Cancer Translational Research Laboratory, Institut Jules Bordet) and colleagues performed a search of gene expression publically available datasets containing patients of known age. A three-gene classifier (ESR1, ERBB2, AURKA) was used to assign patients to BC molecular subtypes [Azim *et al.* 2012]. The difference in RFS was determined using a Cox regression model (stratified by dataset and adjuvant treatment and adjusted for age, BC subtypes, pT, pN and grade). A MEDLINE search on molecular aberrations

related to BC biology in young women was also conducted. The researchers evaluated if gene expression was associated with age, after adjustment, in a linear regression model. Systemically treated and untreated patients were used for the biological analysis and false discovery rate (FDR) was used to control for multiple testing.

There were 3522 eligible patients across 20 datasets. Patients up to 40 years old had a higher proportion of ER-/HER2- tumours but less Luma than their older counterparts ($p < 0.0001$) and were independently associated with poorer RFS (HR 1.34, 95%CI 1.10–1.63, $p = 0.004$). There were 41 genes and 13 gene sets identified as being related to BC biology in young women. In the untreated cohort, the expression of 16 genes and gene sets was found to be significantly related to age after adjustment and 12 were confirmed in the treated cohort. BC arising in younger patients showed signals for biological processes related to immature mammary cell populations (RANKL, c-kit, BRCA1 mutation, mammary stem cells, luminal progenitors cells), and growth factor signalling (mitogen-activated protein kinase, PI3K related); however, it appeared to be associated with downregulation of apoptosis-related genes (FAS, CASP3, BAD).

Thus, BC in young women seems to be a biologically unique cancer beyond subtype distribution, which suggests it may require distinct therapeutic approaches such as RANKL (receptor activator of nuclear factor κ B ligand) inhibition. The authors are continuing their research with a preoperative window study evaluating RANKL inhibition in young patients with BC.

Interleukin 6 and telomere length as markers for ageing and treatment selection in older patients with breast cancer

The potential benefit of treating cancer in older patients needs to be balanced against risks of toxicity and decline in functionality and quality of life. Whilst the Comprehensive Geriatric Assessment (CGA) can reflect biological age to a certain degree, it does have limitations (validity, sensitivity and reliability). Thus, there is a need for better markers of ageing so that selection of patients for therapy can be refined.

With this in mind, Barbara Brouwers (Department of General Medical Oncology – Laboratory of Experimental Oncology, University Hospitals

Leuven – Catholic University Leuven, Leuven, Belgium) and colleagues conducted a retrospective analysis of a panel of biomarkers [circulating levels of interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) (CCL2), RANTES (CCL5) and mean leukocyte telomere length] in blood samples collected at diagnosis from 162 patients with BC (≥ 70 years of age) in whom CGA had also been simultaneously performed [Brouwers *et al.* 2012]. There were three patient categories (fit, vulnerable, frail).

The mean levels of circulating IL-6 correlated significantly with levels of frailty; the mean telomere length tended to decrease with increasing frailty; there was no correlation between frailty and mean circulating levels of MCP-1 or RANTES.

The authors concluded that circulating IL-6 plasma levels correlate significantly with the level of frailty determined by CGA in older patients with BC. They suggest that IL-6 and telomere length need further investigation as markers of biological age in patients with cancer and as potential predictors for treatment toxicity and outcome.

Novel RNA test can filter out patients who are unlikely to respond to chemotherapy

The RNA Disruption Assay (RDA) was developed in the MA-22 trial for women with locally advanced BC as a means of assessing drug efficacy during treatment. In the trial, docetaxel and epirubicin were given at 2-weekly (dose dense) or 3-weekly intervals and tumour biopsies carried out pre therapy, mid therapy and post therapy. Endpoints included clinical response, pCR, DFS and OS. The principle behind the assay is that tumours responding to treatment at mid therapy produce a marked reduction in RNA quality and an algorithm is used to discern between patients who subsequently exhibit a pCR and those who do not: a high RDA score indicates subsequent pCR and a low score indicates no long-term benefit from chemotherapy.

Maureen Trudeau (Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada) presented details from the MA-22 trial showing that after three out of four cycles of chemotherapy in the 85 patients studied, the RDA predicted 32% to be nonresponders (negative predictive value 0.99, CI 0.98–1.0, false-negative rate 2%) [Trudeau *et al.* 2012]. RDA predicted

75% of patients who subsequently achieved a pCR would have an increased chance of responding (positive predictive value 0.17); thus, some patients responded in some way to therapy but did not achieve a pCR.

The authors believe that the RDA has clinical utility as well as cost savings in that it will enable physicians to identify patients who are unlikely to respond to BC chemotherapy: such patients may then be able to avoid the toxicities associated with chemotherapy and can be given alternative treatment options.

High-risk status according to Recurrence Score predicts high risk by uPA/PAI-1, high grade by central pathology, and luminal B subtype by Ki-67

Dr H.H. Gluz [Scientific Development, West German Study Group (WSG), Monchengladbach, Germany) presented a final WSG-Plan B correlation analysis of risk assessment tools with a focus on the correlation between central and local pathology [Gluz *et al.* 2012].

The St Gallen Consensus recommends use of Ki-67 or grade to define LumA/B for adjuvant chemotherapy in HR+ BC. Multi-gene-based assays such as the Recurrence Score (RS) may be used as a decision tool for initiating such adjuvant therapy.

The Plan B trial involved randomization between six cycles of docetaxel–cyclophosphamide chemotherapy and four cycles of epirubicin/cyclophosphamide, which was followed by four cycles of docetaxel in locally HER2– BC. The RS was used as selection criteria for chemotherapy *versus* endocrine therapy alone in HR+ disease (if recurrence score was < 11 in pN0 or pN1). Central grade, ER, PR, HER2 and Ki-67 were evaluated by a central pathologist.

There were 3196 patients recruited, 2448 were randomized and RS was available in 2551 patients with HR+ tumours. The RS was distributed as follows: 0–11 (18%), 12–25 (60%), >25 (22%). Low RS in 354 patients resulted in them being omitted from any chemotherapy. Central grade was available for 3038 samples and IHC results are based on 1476 available cases. Moderate significant correlations were found between RS and central grade, local grade and Ki-67 assessment due to weak correlation within low and intermediate risk groups. Weak

correlation was found for urokinase-type plasminogen activator (uPA)/plasminogen activator inhibitor 1 (PAI-1) and RS. Fifty-five percent of patients were defined as LumB (using a Ki-67 cutoff of 14%) in whom chemotherapy was indicated. Local and central grade showed a 68% concordance, 57% of tumours classified as G3 by a local board of certificated pathologists were G3 by central assessment.

These are the first prospective data to show that high-risk status according to RS is predictive of high risk by uPA/PAI-1, high grade by central pathology and LumB subtype by Ki-67. There was substantial heterogeneity in risk assessment in the low and intermediate risk RS groups in which some patients are still considered to be high risk according to uPA/PAI-1, Ki-67 or central grade. The authors stated that follow up of the WSG-Plan B trial will clarify the significance of these findings with regard to patient outcomes.

Cost effectiveness of Oncotype DX in breast cancer

The Oncotype DX® (ODX, Genomic Health Inc., Redwood, CA, USA) 21 gene assay can be used to determine personalized treatment in early-stage, node-negative, ER+ BC. Its cost effectiveness has been assessed in multiple studies and Drs P. Pronzato and J.A. Plun-Favreau (Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy and Health Economics, Genomic Health, Geneva, Switzerland) have reviewed the consistency of methods and findings from published studies [Pronzato and Plun-Favreau, 2012]. PubMed and some congress databases were searched for cost-effectiveness analyses of ODX and were subjected to methodological quality assessment. A total of five published health economics analyses and six abstracts were identified from countries including the USA, Canada, Japan, Israel, Singapore, UK, Germany, France and Hungary. Markov modelling was used to make estimates of long-term outcomes and assess the cost effectiveness of using the ODX recurrence score. Adjustments were made for each individual country.

Their results showed that in the USA and France, ODX use was cost saving; however, in one of the Canadian studies it was cost effective (incremental cost-effectiveness ratio of US\$64,063 per quality-adjusted life year gained). The authors concluded that the currently published literature is of good

methodological quality and consistently supports the cost effectiveness of using ODX in various settings. They suggest that further analyses should be performed to evaluate the budget impact of funding ODX and to include a broader perspective of the costs.

Roman Rouzier (Gynecology, Tenon, Paris, France) and colleagues presented the results of their new study on the long-term cost effectiveness of the ODX assay in French clinical practice [Rouzier *et al.* 2012]. They developed a Markov model to evaluate long-term costs and clinical outcomes as a result of using the ODX to assist with decisions on adjuvant chemotherapy in ER+, node-negative early-stage BC. The model projected life expectancy and costs based on recurrence rates for low-, intermediate- and high-risk patients, and on French mortality data. It compared the assignment of adjuvant chemotherapy (conventional approach *versus* ODX RS) using data from nine studies. Costs of chemotherapy were collected retrospectively at the hospital and costs of long-term recurrence were collected from published literature.

The researchers found that, compared with current clinical practice, using ODX decreased chemotherapy cost by €630 per patient (e.g. by sparing patients from unnecessary chemotherapy and by increasing outcomes). Thus, ODX is expected to be cost saving in French clinical practice.

Molecular biology

Inhibition of the transcription factor activator protein 1 potentiates the effect of endocrine treatment and circumvents endocrine resistance

L. Malorni (Oncology, Hospital of Prato, Prato, Italy) and colleagues have previously shown that acquired endocrine resistance is associated with increased activator protein 1 [AP-1, a transcription factor downstream of different growth factor receptors (GFRs)] activity and that AP-1 modulates the ER transcriptional programme, especially with high GFR signalling [Malorni *et al.* 2012]. Thus, AP-1 modulation may circumvent endocrine resistance.

To study this, the researchers conducted *in vitro* studies and showed that siRNA c-Jun (used to genetically inhibit AP-1) significantly inhibited the growth of acquired tamoxifen resistance (TamR) in MCF7 cells but not in parental cells.

Mice with xenografts of two inducible dominant-negative (DN) c-Jun clones were randomized to continued estrogen supplementation or to either estrogen deprivation or tamoxifen, in the presence or absence of DN c-Jun. Blocking AP-1 was found to significantly reduce time to tumour response and disappearance in the tamoxifen group, with similar results in the estrogen deprivation group, and it also significantly delayed TamR. DN c-Jun induction produced dramatic tumour shrinkage after long-term tamoxifen treatment, which suggests a reversal of endocrine resistance with AP-1 blockade. Thus, AP-1 inhibition potentiates the effect of endocrine treatment and circumvents endocrine resistance. Drugs with AP-1 inhibitory activity may represent a novel therapeutic strategy to overcome endocrine resistance and improve outcome in patients with BC.

Drug developments

PD 0332991 plus letrozole compared with letrozole alone improves progression-free survival in patients with ER+/HER2- breast cancer

PD 0332991 is an oral, highly selective inhibitor of cyclin dependant kinase 4/6 activity that prevents cellular DNA synthesis by prohibiting progression of the cell cycle from G1 to S phase. In preclinical studies luminal ER+ and HER2 amplified subtypes, elevated expression of cyclin D1 and Rb protein, and reduced p16 expression were identified as being associated with sensitivity to PD 0332991. Synergistic activity with tamoxifen was also observed *in vitro*. Based on these observations, a phase I/II study in combination with letrozole was initiated. The phase I study determined the daily dosage as letrozole 2.5 mg daily plus PD 0332991 125 mg daily on Schedule 3/1. Richard Finn (Department of Hematology and Oncology, University of California, Los Angeles, CA, USA) and colleagues presented results from the first part of a randomized phase II study [Finn *et al.* 2012]. The study population comprised 66 postmenopausal women with ER+/HER2- breast cancer who were randomized to PD 0332991 plus letrozole or letrozole 2.5 mg daily alone. The primary endpoint was progression-free survival (PFS) and secondary endpoints included response rate, OS, safety and correlative biomarker studies. Patients continued with their assigned study treatment until disease progression, unacceptable toxicity or consent withdrawal, and were followed for tumour assessments every 2 months.

The median duration of treatment was 47 weeks for the PD 0332991 plus letrozole arm and 24 weeks for the letrozole monotherapy arm. The objective response rate was 52% *versus* 32% (out of 27 and 22 patients, PD 0332991 plus letrozole *versus* letrozole, respectively), clinical benefit rate (partial response plus stable disease) was 76% *versus* 47%, respectively. PFS was significantly greater with PD 0332991 plus letrozole than letrozole alone (HR 0.35; 95% CI 0.17–0.72, $p = 0.005$). The most commonly reported treatment-related adverse events in the combination arm were neutropenia, leukopenia and fatigue.

The authors concluded that the combination of PD 0332991 plus letrozole compared with letrozole alone showed statistical improvements in median PFS in patients with ER+/HER2– BC, confirming the preferential sensitivity of ER+ BC in preclinical models. The use of exploratory biomarkers for cyclin D1 gains or p16 loss did not add to ER+ alone as a selection marker for patients who may benefit from PD 0332991. A phase III study is being planned.

NKTR-102 has significant antitumour activity in patients with advanced metastatic breast cancer

Ahmad Awada (Medical Oncology Clinic, Institut Jules Bordet) and colleagues conducted an open-label phase II study in 70 patients with advanced metastatic BC (median age 55 years; range 37–83 years; Eastern Cooperative Oncology Group Performance Status 0/1 40%/60%; 61% ER/PR+; 30% triple-negative BC, 86% visceral metastases) who were randomized to the topoisomerase I inhibitor-polymer conjugate, NKTR-102 (145 mg/m² intravenously over 90 min) every 14 or 21 days) [Awada *et al.* 2012]. The primary endpoint was objective response rate (ORR) by RECIST v1.0, and secondary endpoints were PFS and safety. Patients had received a median number of two prior cytotoxic regimens for metastatic BC: all had received prior taxane therapy and 89% had received prior anthracyclines.

For the total population, the ORR over 14 or 21 days was 32% and 26%, respectively. ORRs were also calculated for subgroups that had received prior chemotherapy (33% and 30%, respectively), had triple-negative BC (25% and 50%) or visceral disease (32% and 28%). The median PFS was 3.5 months and 5.3 months for the 14- and 21-day schedules, respectively.

Common related grade 3/4 toxicities included diarrhoea in about 20% of patients, neutropenia (11%), fatigue (about 10%) and dehydration (about 10%). There were 20% and 14% withdrawals per 14- and 21-day schedules.

The authors concluded that NKTR-102 has significant antitumour activity in patients with advanced metastatic BC, including those with visceral disease or prior chemotherapy. An international phase III clinical trial comparing single-agent NKTR-102 to physician's choice of treatment is underway.

Everolimus plus exemestane is a promising therapeutic option for patients with ER+ advanced breast cancer

Hope Rugo (Department of Medicine, University of California, San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA) presented updated results of the phase III BOLERO-2 trial (one of a series of Breast Cancer Trials of Oral Everolimus) looking at the addition of everolimus for postmenopausal women with advanced BC [Rugo *et al.* 2012]. (The preplanned interim analysis was presented at the 16th European Cancer Organisation–36th European Society for Medical Oncology Multi-disciplinary Congress, Stockholm, 2011.)

This double-blind, placebo-controlled study evaluated everolimus plus exemestane in 724 patients with ER+ advanced BC who were refractory to the AIs, letrozole or anastrozole. Patients were randomized (2:1) to exemestane (25 mg/day) plus everolimus (10 mg/day) or placebo. The primary endpoint was PFS with secondary endpoints of OS, response rate, quality of life (QoL), and safety. Their latest analysis included 457 events and a median follow up of 12.5 months.

Median PFS (investigator assessment) was 7.4 for everolimus *versus* 3.2 months for placebo ($p < 1 \times 10^{-16}$; HR 0.44, 95% CI 0.36–0.53). Median PFS (central assessment) was 11.0 months for everolimus *versus* 4.1 months for placebo ($p < 1 \times 10^{-16}$; HR 0.36, 95% CI 0.28–0.45). Response and clinical benefit rates were also higher for everolimus compared with placebo. There have been 138 deaths: 17.3% in the everolimus group and 22.6% in the placebo group. The most common grade 3/4 adverse events for the everolimus and placebo groups, respectively, were stomatitis (8% *versus* 1%), anaemia (7% *versus* 1%), hyperglycaemia

(5% *versus* < 1%), dyspnoea (4% *versus* 1%) and fatigue (4% *versus* 1%). A grade 3 pneumonitis was observed in 3% of patients on everolimus.

The authors concluded that everolimus plus exemestane produces a significant and sustained extension of PFS in patients with advanced BC refractory to letrozole or anastrozole. Adverse events were higher among patients on everolimus but they were manageable and did not affect QoL. Thus, the drug combination is a promising therapeutic option for patients with ER+ advanced BC.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

- Awada, A., Chan, S., Jerusalem, G.H.M., Coleman, R.E., Huizing, M.T. and Mehdi, A. (2012) Significant antitumor activity in a randomized phase 2 study comparing 2 schedules of NKTR-102 in patients (pts) with metastatic breast cancer (MBC). *Ann Oncol* 23(Suppl. 2): ii43.
- Azim, H.J. Jr, Michiels, S., Bedard, P.L., Singhal, S.K., Criscitiello, C., Ignatiadis, M. *et al.* (2012) Biology of breast cancer diagnosed in young women: pooled gene expression analysis from 3522 patients. *Ann Oncol* 23(Suppl. 2): ii19.
- Bachelot, T., Bourcier, C., Cropet, C., Guastalla, J.-P., Ferrero, J.-M., Leger-Falandry, C. *et al.* (2010) TAMRAD: A GINECO randomized phase II trial of Everolimus in combination with Tamoxifen versus Tamoxifen alone in patients (pts) with hormone-receptor positive, HER2 negative metastatic breast cancer (MBC) with prior exposure to Aromatase inhibitors (AI). *Cancer Res* 70(Suppl. 2): abstract S1-6.
- Bachelot, T., Ferrero, J., Cropet, C., Bourcier, C., Abadie-Lacourtoisie, S., Levy, C. *et al.* (2012) Translational studies within the TAMRAD randomized GINECO trial: evidence for TORC1 activation marker as a predictive factor for everolimus efficacy in metastatic breast cancer (mBC). *Ann Oncol* 23(Suppl. 2): ii18.
- Bozovic, I., de Azambuja, E., Loi, S., Ameye, L. and Piccart, M. (2012) Bcl2 negative breast tumors but not axillary lymph nodes predict a better overall survival in patients treated with higher doses of epirubicin at a median follow-up of 15.6 years. *Ann Oncol* 23(Suppl. 2): ii21.
- Brouwers, B., Hatse, S., Corthouts, K., Swerts, E., Kenis, C., Neven, P. *et al.* (2012) Ageing biomarkers in an elderly breast cancer population: correlation with comprehensive geriatric assessment (CGA). *Ann Oncol* 23(Suppl. 2): ii23.
- Dowsett, M., Procter, M., de Azambuja, E., Scullion, M.J., Gelber, R.D. and Leyland-Jones, B. (2012) HER2 extracellular domain levels in patients with HER2-positive early breast cancer experiencing disease progression: case-control comparison of samples from the HERA study. *Ann Oncol* 23(Suppl. 2): ii20.
- Finn, R.S., Crown, J.P., Boer, K., Lang, I., Parikh, R.J., Breazna, A. *et al.* (2012) Results of a randomized phase 2 study of PD 0332991, a cyclin-dependent kinase (CDK) 4/6 inhibitor, in combination with letrozole vs letrozole alone for first-line treatment of ER+ /HER2- advanced breast cancer (BC). *Ann Oncol* 23(Suppl. 2): ii43.
- Fumagalli, D., Lambrechts, D., Neven, P., Vanderhaegen, J., Claes, B., Singhal, S.K. *et al.* (2012) Molecular characterization of early-stage HER2-overexpressing breast cancer (HER2+ BC) treated with adjuvant trastuzumab: identification of prognostic groups. *Ann Oncol* 23(Suppl. 2): ii21.
- Gijssen, M., Tang, P., Leung, W.Y., Roxanis, I. and Kong, A. (2012) PTPN9 is a negative regulator of HER3 phosphorylation and a prognostic biomarker in HER2 positive breast cancer. *Ann Oncol* 23(Suppl. 2): ii21.
- Gluz, O., Kreipe, H.H., Degenhardt, T., Salem, M., Kates, R.E., Shak, S. *et al.* (2012) Prospective comparison of risk assessment tools in early breast cancer: correlation analysis from the phase III WSG-Plan B trial. *Ann Oncol* 23(Suppl. 2): ii18.
- Ignatiadis, M., Singhal, S.K., Desmedt, C., Haibe-Kains, B., Criscitiello, C. and Andre, F. (2012) Gene modules and pathological complete response to neoadjuvant chemotherapy in breast cancer. A pooled analysis. *Ann Oncol* 23(Suppl. 2): ii17.
- Malorni, L., Giuliano, M., Migliaccio, I., Creighton, C., Lupien, M., Hilsenbeck, S. *et al.* (2012) The role of AP-1 in endocrine-resistant breast cancer. *Ann Oncol* 23 (Suppl. 2): ii37.
- Prat, A., Cheang, M.C., Martin, M., Carrasco, E., Caballero, R., Tyldesley, S. *et al.* (2012) Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically-defined luminal a breast cancer. *Ann Oncol* 23(Suppl. 2): ii17.
- Pronzato, P. and Plun-Favreau, J.A. (2012) Cost-effectiveness review of the 21-gene breast cancer test. *Ann Oncol* 23(Suppl. 2): ii22.

Rouzier, R., Chereau, E., Laas, E., Genin, A., Bendifallah, S. and Gligorov, J. (2012) Cost-effectiveness evaluation of the 21-gene breast cancer test in France. *Ann Oncol* 23(Suppl. 2): ii28.

Rugo, H., Piccart, M., Hortobagyi, G., Noguchi, S., Gnant, M., Campone, M. *et al.* (2012) Updated results of the BOLERO-2 phase III trial evaluating everolimus (EVE) for postmenopausal women with advanced breast cancer (ABC). *Ann Oncol* 23(Suppl. 2): ii46.

Sinn, B.V., von Minckwitz, G., Denkert, C., Eidtmann, H., Darb-Esfahani, S., Belau, A. *et al.*

(2012) Mucin-1 protein and mRNA expression in breast cancer patients treated with neoadjuvant chemotherapy. *Ann Oncol* 23(Suppl. 2): ii17.

Trudeau, M.E., Pritzker, L.B., Parissenti, A.M., Wang, X., Zhu, M., Guo, B. *et al.* (2012) A novel RNA test to guide primary systemic breast cancer chemotherapy. *Ann Oncol* 23(Suppl. 2): ii19.

von Minckwitz, G., Kümmel, S., Vogel, P., Hanusch, C., Eidtmann, H., Hilfrich, J. *et al.* (2008) Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomized GeparTrio study. *J Natl Cancer Inst* 100: 552–562.