Advancing breast cancer therapy by translational research: highlights of the Improving Care and Knowledge through Translational Research (IMPAKT) breast cancer conference

Ther Adv Med Oncol

(2011) 3(5) 223–227

DOI: 10.1177/

1758834011413003

© The Author(s), 2011.
Reprints and permissions:
[http://www.sagepub.co.uk/
journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

Gordon Mallarkey and R. Charles Coombes

Researchers and clinicians attended the IMPAKT (Improving Care and Knowledge through Translational Research) breast cancer conference in Brussels, Belgium (5–7 May 2011). The aim of the conference was to bring together researchers and clinicians interested in advancing breast cancer therapy by cutting-edge translational research. The biological heterogeneity of breast cancer means that therapeutic advances will happen only if synergies between translational and clinical research can be established and utilized. This editorial features highlights of the ‘Best Abstracts’ session held at the conference.

Celecoxib has antitumor activity in breast cancer

The results of a randomized trial in 45 patients with primary invasive breast cancer showed that the cyclo-oxygenase-2 (COX-2) drug, celecoxib, induces an antitumor response at the molecular level. This confirms existing data from several preclinical studies where COX-2 inhibition led to changes in cell proliferation, apoptosis, and extracellular matrix biology in primary breast cancer tissues.

Juergen Veeck (Maastricht University Medical Centre, The Netherlands) and colleagues studied 45 patients with primary invasive breast cancer who were scheduled to have surgery to remove their cancer. Prior to surgery, patients were

randomly assigned to receive celecoxib 400 mg twice daily for 2–3 weeks or control treatment. Gene expression profiling using Affymetrix U133 Plus 2.0 arrays were performed on core biopsies before treatment and surgical resections specimens after treatment. Bio-informatic tools were used to identify significantly altered pathways after celecoxib treatment; immunohistochemical Ki67 and caspase-3 staining were performed on all tissue sections to determine changes in proliferation and apoptosis, respectively.

After treatment, 1109 genes were significantly upregulated and 556 genes significantly downregulated in celecoxib-treated breast cancer tissues when compared with the control treatment. Pathway analyses showed that genes involved in cell proliferation, cell cycle, apoptosis, extracellular matrix biology, and inflammatory immune responses were particularly affected [Veeck *et al.* 2011].

Thus, short-term treatment with celecoxib appears to set up transcriptional programs that support antitumor activity in primary breast cancer tissues. The next step is to find out if longer treatment with celecoxib results in measurable tumor shrinkage. Tumor models have shown there are close links between COX-2 expression and HER2 status and aromatase levels in breast cancer; thus, it may be worth investigating COX-2 inhibitors in combination

Correspondence to:
Dr Gordon Mallarkey
Sage Publications Ltd,
1 Oliver's Yard, 55 City
Road, London EC1Y 1SP,
UK
[mallarkey@
sagepub.co.uk](mailto:mallarkey@sagepub.co.uk)

Prof. Charles Coombes
Director of Cancer
Research UK
Laboratories, Head of
Department of Oncology,
Imperial College,
Hammersmith Hospital
Campus, Du Cane Road,
London, UK

with trastuzumab or aromatase inhibitors in the clinical setting.

Prospective study shows prognostic value of circulating tumor cells in breast cancer

Circulating tumor cell (CTC) counts during treatment have been reported as an independent prognostic factor in metastatic breast cancer (MBC); however, data comparing CTCs with serum tumor markers are limited. Dr François-Clement Bidard and colleagues (Institut Curie, Paris, France) conducted a prospective study to validate these markers in a large series of patients receiving first-line chemotherapy for MBC including assessment of usual serum markers and CYFRA 21-1 [Bidard *et al.* 2011].

Their prospective study included 267 patients who were receiving first-line chemotherapy for MBC. A CTC count plus an analysis of other blood markers was performed in each patient, from 7.5 ml blood samples taken at the start of treatment and at several later time points thereafter (before cycle 2, at clinical and/or radiological evaluation [C3–C4], and at tumor progression). Analysis was performed centrally using the CellSearch® System. Median follow-up was 16 months: 39 patients (15%) had HER2+ tumors, 55 (21%) had triple-negative breast cancer (TNBC).

Of 260 evaluable patients, 170 (65%) had at least one CTC per blood sample at baseline, and 115 (44%) had five or more CTCs. Multivariate analysis showed that TNBC, performance status (PS), CTC, and carcino-embryonic antigen were prognostic for progression-free survival (PFS), and TNBC, CTC, and PS for overall survival. CYFRA 21-1 was associated with PFS ($p=0.05$) in multivariate analysis when added to these models.

This is the first prospective study that has been designed and statistically powered for reporting CTC-associated outcome as a primary endpoint in a homogeneous population of MBC patients treated first line. The study results help answer the question about how many CTCs per blood sample should be used to define patients at 'high risk' for a poor outcome. While it appears that poor outcome is determined by the more CTCs per blood sample, a threshold still needs to be defined. The relative risk of the high-risk group *versus* low-risk group did not significantly change whether it was defined by a threshold of 1 CTC

or 5 CTCs: in both cases, the relative risk of a shorter PFS in the high-risk group compared with the low-risk group was statistically the same. Thus, it appears that using a lower threshold of 1 CTC is feasible without major loss of specificity of CTC detection.

Dr Bidard reported that they are now certain that CTCs are prognostic at baseline, and that CTC changes under treatment may be an early indicator of chemotherapy efficiency. He believes that future interventional randomized trials in MBC should try to demonstrate that CTC-based treatment strategies lead to better clinical outcomes, and/or at least to an improvement in the cost/efficacy ratio of treatment. Interventional trials (SWOG0500 and CirCe01) to assess the use of CTC to modify treatment are ongoing.

Epigenetic study reveals new insights into breast cancer

Epigenetics describes modifications to the DNA molecule that affect the way its code is translated into proteins. These changes include methylation, a form of chemical modification. Researchers have known that epigenetics is important in cancer, but information about its exact contribution to breast carcinogenesis has been scarce. The most comprehensive analysis yet of the epigenetic modifications present in breast cancer has revealed potentially important new ways to detect and treat the disease.

Dr Sarah Dedeurwaerder and colleagues (Université Libre de Bruxelles, Brussels, Belgium) have assessed the epigenetic differences between normal tissue and primary tumor samples on a genome-wide scale, similar to that done for gene expression patterns. Their results provide a new insight into what DNA methylation profiling of primary breast tumors might bring towards an understanding of their biology and diversity, which could lead to better management of breast cancer patients [Dedeurwaerder *et al.* 2011].

They performed a comprehensive DNA methylation profile on two independent sets of frozen breast tissue samples: a 'main set' of 123 samples, (4 normal and 119 infiltrating ductal carcinomas), and a 'validation set' of 125 samples (8 normal and 117 infiltrating ductal carcinomas). They used Illumina's Infinium Methylation Assay, which allows assessment of the

methylation status of more than 27,000 CpGs corresponding to over 14,000 genes.

They found that the two major subtypes of breast cancer, defined by estrogen receptor (ER) status, are widely epigenetically controlled. Clustering analysis of samples, based on their DNA methylation profiles, showed that tumors segregated naturally into two distinct groups: the first group mainly composed of ER-negative tumors, the second one of ER-positive tumors, indicating that ER-negative and ER-positive tumors have very different methylation profiles.

They also looked at more than 400 genes whose expression was positively or negatively correlated to the expression of the ER gene and showed a reverse correlation between methylation and expression status of the majority of these genes, suggesting that epigenetics is probably involved in the regulation of expression of genes and plays an important role in the establishment of the two major phenotypes of breast cancer determined by ER status.

Their analysis also provided interesting new information about new subtypes of breast cancer: they showed that DNA methylation profiles enabled breast tumors to be classified in more groups than those currently defined. Several patients displaying the same known subtype of breast cancer may respond differently to a given drug – an epigenetic difference between the tumors in such patients may explain differences in treatment response. Thus, DNA methylation profiling has the potential to help refine current breast cancer classification and so lead to stratification of patients within a particular subtype, both in terms of prognosis and prediction of treatment response.

Dr Dedeurwaerder believes that epigenetic information may be utilized to improve cancer care as follows.

1. There is evidence showing that epigenetic dysregulation may occur early during carcinogenesis and can be detected in bodily fluids. Therefore, DNA methylation markers could help with earlier detection of the disease.
2. It has already been shown that DNA methylation markers might help to better stratify patients in terms of prognosis.

3. Such markers could also help to predict response to a given drug.
4. Epigenetic therapy of cancer, alone or in combination with conventional therapies, is conceivable.

Comparison of breast cancer multigene tests

Several prognostic genomic tests have been developed for breast cancer. Dr Catherine Kelly (Mater Misericordiae University Hospital, Dublin, Ireland) has looked at the agreement in prediction results between two multigene assays: Oncotype DX *versus* PAM50, which is undergoing clinical evaluation.

Oncotype DX measures the activity of 21 genes and generates a recurrence score (RS) that categorizes patients into low, intermediate, or high-risk groups depending on the risk of distant recurrence. It is intended for use in patients with ER-positive, lymph node-negative breast cancer to identify women at low risk of breast cancer recurrence who safely can avoid chemotherapy. PAM50 is a breast cancer intrinsic classifier and measures the expression of 50 genes then stratifies breast cancers into five subtypes: luminal-A, luminal-B, basal-like, HER2-enriched and normal-like.

Dr Kelly's team assessed the two tests on 119 breast cancer specimens from patients classified as being at 'clinically intermediate' risk for recurrence based on the following criteria: median tumor size 1.5 cm, all ER-positive, HER2-negative, lymph-node negative, and most grade II. In this group of patients, it is difficult to determine whether or not they would benefit from chemotherapy; thus, multigene assays may provide additional independent prognostic information.

The results showed that of the 10 patients with high RS (Oncotype DX), nine were classified as luminal-B and one as basal-like by the PAM50 classifier; however, 53 out of 64 low RS cases were luminal-A type. There were 23 of 45 intermediate RS cancers that were re-categorized as low-risk luminal-A cancers by PAM50. All luminal-A cancers were either low (53) or intermediate (23) risk by RS, whereas luminal-B cancers spanned all Oncotype DX risk groups [Kelly *et al.* 2011].

Thus, for high and low prognostic risk assignment, the two methods showed reasonably good

agreement for high and low prognostic risk assignment, but this was not the case for intermediate risk groups.

Dr Kelly said that it is uncertain if patients with an intermediate Oncotype DX RS benefit from chemotherapy. The fact that PAM50 re-categorized about half of the patients with intermediate RS to the low-risk luminal-A category suggests that these patients may not benefit much from adjuvant chemotherapy due to their already very good prognosis and limited chemotherapy sensitivity. Validation of these findings in a larger population is mandatory before they can be translated into clinical practice.

Gene expression reveals a molecular marker that may help predict chemotherapy response in TNBC

An unexpected molecular marker has been identified that predicts how sensitive hard-to-treat TNBCs are to chemotherapy. Carolin Huelseswig and Dr Cornelia Liedtke (University of Muenster, Germany) reported that the molecule sFRP1 is much more highly expressed in these cancers, and that levels of the molecule in an individual tumor correlate with its sensitivity to chemotherapy [Huelseswig *et al.* 2011].

The first step of the study was to conduct gene expression analysis using Affymetrix U133A gene chip data in breast cancer tissue samples, looking specifically for genes differentially expressed between TNBCs and non-TNBCs treated with neo-adjuvant taxane/anthracycline chemotherapy. This showed that sFRP1 was the most highly overexpressed gene in TNBCs by up to 4.7-fold *versus* non-TNBCs. This was a surprise finding as sFRP1 has so far been understood as an antagonist within the Wnt signaling cascade.

The researchers then tested genes for an association with relapse-free survival, response to neo-adjuvant chemotherapy and correlation with Ki67 expression. Whilst sFRP1 expression was not associated with recurrence-free survival in TNBC, they found it was significantly correlated with an increased sensitivity to neo-adjuvant chemotherapy. No correlation between expression of Ki67 and sFRP1 was found.

The final part of the study consisted of 'knock-down' experiments in cell culture, using the TNBC cell line, MDA-MB 468, and involved

using short segments of RNA (siRNA) designed to block the expression of sFRP1. Significantly decreased sensitivity to paclitaxel, doxorubicin, and cisplatin was found in breast cancer cells where sFRP1 expression was knocked down.

Thus, the results suggest that sFRP1 is a novel predictive biomarker tailored to the TNBC subtype. This study provides a 'proof-of-principle' that identification, validation and functional analysis of biomarkers for specific disease subtypes are feasible through translational research incorporating both *in silico* analyses, such as gene expression profiling, and basic science including functional analyses.

HER2/HER3 protein interactions reveal clues to breast cancer outcomes

Dr Gargi Patel (Richard Dumbleby Department, King's College London, UK) reported results from a microscope technique known as Förster resonance energy transfer imaging, which allows them to measure protein-protein interactions [Patel *et al.* 2011]. Each protein is labeled with a fluorescent tag, which is excited by a laser. When two proteins are close enough for interaction, some energy is transferred from one label to the other and the fluorescent lifetime is shortened. Thus, the technique can be used to quantify protein-protein interactions.

This technique has already been used by Dr Patel's group on breast cancer cells to characterize the molecular determinants for lapatinib-responsive formation of the HER2/HER3 complex. They have identified a specific HER2 mutation that reduces dimerization and lapatinib's effect. Thus, tumor testing for this HER2 mutation would help to identify resistance to treatment. Current methods of prognosis estimation rely on clinical and genetic data, however, no single method is 100% accurate. Dr Patel's aim is to add their system of measuring protein-protein interactions to the tools available to predict more accurately outcomes.

References

Bidard, F., Hajage, D., Bachelot, T., André, F., Brain, E.G., Campone, M. *et al.* (2011) Circulating tumor cells and Cyfra 2-1 as outcome-associated biomarkers in first-line metastatic breast cancer, results of the IC 2006-04 study. *Ann Oncol* 22(Suppl 2): ii41.

Dedeurwaerder, S., Desmedt, C., Calonne, E., Singhal, S.K., Haibe-Kains, B., Defrance, M. *et al.* (2011) Epigenetic portraits in breast cancers. *Ann Oncol* 22(Suppl 2): ii29.

Huelsewig, C., Ruckert, C., Goette, M., von Wahlde, M.K., Buchweitz, O., Kiesel, L. *et al.* (2011) The wnt signaling cascade as a potential biomarker of triple-negative breast cancer (TNBC). *Ann Oncol* 22(Suppl 2): ii51.

Kelly, C.M., Bernard, P.S., Krishnamurthy, S., Ebbert, M.T.W., Bastien, R.R.L., Boucher, K. *et al.* (2011) Concordance between the 21 gene assay (OncotypeDX[®]) and the PAM50 intrinsic classifier for

prognosis in early stage ER-positive breast cancer. *Ann Oncol* 22(Suppl 2): ii44.

Patel, G., Lawler, K., Weitsman, G., Fruhwirth, G., Woodman, N., Gillett, C. *et al.* (2011) Combining protein interaction and gene profiling methods for predicting lapatinib response. *Ann Oncol* 22(Suppl 2): ii43.

Veeck, J., Brandao, R.D., van Elssen, J.C., van de Vijver, K.K., Lindsey, P.J., Blok, M.J. *et al.* (2011) A randomized trial of pre-operative celecoxib treatment reveals anti-tumor transcriptional response in primary breast cancer. *Ann Oncol* 22(Suppl 2): ii44.

Visit SAGE journals online
<http://tam.sagepub.com>

SAGE JOURNAL
Online