Biomarkers in Breast Cancer in the era of Molecular Pathology

Fernando C. Schmitt, MD, PhD, FIAC
Associate Professor of Pathology, Medical Faculty of Porto University
Director of the Unit of Molecular Pathology – IPATIMUP

e-mail: fernando.schmitt@ipatimup.pt

Breast cancer is the most common non-cutaneous malignancy in women in the Western world, being the lifetime risk of developing an invasive breast carcinoma in the USA around 12.03% (1 in every 8 women). By itself it is expected to account for 27% of all new cancer cases among women. It is a very heterogeneous disease, encompassing numerous entities that present not only biological differences but also a diverse clinical behavior. The current clinical management still relies on traditional prognostic and predictive factors, like, histology, clinical and well defined biologic factors like, estrogen and progesterone receptors (ER and PR), as well as the human epidermal growth factor receptor 2 (HER2), all of which present an association with prognosis and treatment outcome. However, this classification system fails at taking into account the tumor heterogeneity, as even tumors that apparently present the same characteristics, can have markedly different responses to therapy and present distinct outcomes. The use of high throughput molecular technologies has enabled the better understanding of this complexity, by allowing the classification of breast tumors into biologically and clinically distinct groups based on their gene expression patterns. Noteworthy, this classification in molecular subtypes also presents a predictive value. From the point of view of treatment, breast cancer patients fall into three categories: the hormone receptor positive that can be treated with estrogen receptor target therapies with or without adding chemotherapy; the HER2 positive that will receive HER2-directed therapy either with the monoclonal antibody trastuzumab or the tyrosine kinase inhibitor lapatinib; or those that are negative for hormone receptors and HER2 that are solely treated with chemotherapy. The expression of oestrogen receptor (ER) is an important prognostic and predictive factor in breast cancer and has relevant implications for the biology of this type of carcinomas. Patients with tumours that express ER have a longer disease-free interval and overall survival than patients with tumourslacking ER expression. In fact these tumours are not homogenous and can be divided at least in two types: luminal A and luminal B, based on the co-expression of HER2 or in the high proliferative index. This subdivision is important in a therapeutic point of view because the luminal B tumours are more aggressive, more frequently develop resistance and should be treated with chemotherapy. Molecular genetics signatures have been used to detect ER aggressive tumours and at least two commercial tests are in use: Oncotype DX and Mammaprint. Using different approaches these tests are only clinically valid for ER positive cases since most of ER negative cases have bad genomic signature. A word of caution should be raised concerning these tests because as was recently demonstrated their reproducibility are not yet ideal and more standardization is needed. Moreover, we need to look not only for the tumour but also for the patient and polymorphisms in genes of enzymes that metabolize drugs, such as CYP2D6 can influence the response to tamoxifen for example. At present,

HER2 expression and/or amplification should be evaluated in every primary invasive breast cancer either at the time of diagnosis or at the time of recurrence, mostly to guide selection of trastuzumab in the adjuvant and/or metastatic setting. Despite the rigorous selection of patients that are likely to benefit from trastuzumab therapy, a significant proportion of patients continue to develop tumour recurrence or progression; even if they respond initially, acquired resistance to trastuzumab will frequently develop. Several mechanisms of trastuzumab resistance have been investigated, mostly based on *in vitro* studies, such as PTEN inactivation/loss and mutation in the catalytic PI3KCA subunits, both leading to increased Akt activity, increased signalling from other HER receptors as EGFR, increased signalling of insulin-like growth factor (IGF) receptor, reduced p27, altered receptor–antibody interaction with masking of HER2 by MUC4 and absence or loss of the external domain of the receptor, resulting in the truncated and constitutively activated form of the receptor (p95HER2). So, we are now in a fascinating phase, where the evaluation of one molecular marker may not be sufficient to predict responsiveness to driven therapies. Pathology laboratories should be prepared for further tests, and translational research will have a critical function in determining the individual management of breast cancer samples, as well-designed clinical trials with standardised evaluation of putative molecular markers associated to drug responsiveness will dictate the success of HER2-overexpressing breast cancer treatment. Also, in HER2-positive tumours not only the tumour alterations are important to determine response in therapy, but also polymorphisms in the FC fragment of IgG influences the ADCC mechanism of toxicity of the trastuzumab. Triple-negative breast cancers (TNBC), defined as tumours that are negative for ER,PR and HER2, nowadays represent the focus of increasing interest at the clinical, biological and epidemiological level due to the aggressive behaviour of the tumour, poor prognosis and present lack of targeted therapies. A better understanding of pathological mechanisms of TNBC onset and progression, including the still unclear association withBRCA1mutations, and the causes of phenotypic heterogeneity may allow improvement in planning prevention and designing novel individualized treatments for this breast cancer subgroup. Immunohistochemistry (IHC) is frequently used to explore the distribution of the molecular subtypes by using formalinfixed, paraffin-embedded tissues from larger cohorts of breast cancer patients. The ultimate selection of surrogate markers is an ongoing debate and a consensus for an appropriate panel still has to be reached. Triple negativity is often used to identify basal-like tumors although a supplement of additional markers has superior prognostic value. IHC-based studies use different markers to define their basal-related tumors and the lack of a systematic classification scheme makes comparison of results difficult. Although triple-negativity coupled with positivity for CK5 and/or EGFR are the pane more frequently used, our group recently demonstrated that adding P-Cadherin, Vimentin and CK14, is possible to detect basal-like carcinomas that were negative for CK5 and EGFR. To understand the biology of these tumours are important and our group also showed the role of P-cadherin in induce cell invasion and cell migration in these tumours. This is now more relevant since antibodies anti-P cadherin are now in clinical trials. Other putative target for therapy in TN tumours is EGFR. Increased HER1 expression is detected in about 25% of TN breast carcinomas

(and more than 90% of metaplastic carcinomas), where it possibly substitutes ineffective, but otherwise major proliferation/survival pathways of breast cancer induced by expression and activation of HER2, ER and PR proteins. Currently, however, HER1 gene status is not used in clinical practice to guide therapy in breast cancer. HER1 protein could be targeted by monoclonal antibodies and/or synthetic tyrosine kinase inhibitors (TKIs). Monoclonal antibodies (cetuximab, panitumumab) are now clinically used in the treatment of colorectal cancer and head and neck carcinoma. Given the subset of TNBC which overexpresses EGFR, targeting EGFR seems to be a rational approach. Although cetuximab monotherapy has little clinical activity, in combination with chemotherapy it may enhance tumor response. EGFR targeted treatment with cetuximab in breast cancer has not produced satisfactory results probably because of the activation of downstream signaling pathways or because of inadequate patient selection. Evaluation of the results of studies testing TKIs (erlotinib, gefitinib) in breast cancer indicated that EGFR protein must be present in targeted tumor tissue to obtain valuable treatment results. Furthermore, it might be better to target more than one of these receptors simultaneously. Targeting angiogenesis by the monoclonal antibody bevacizumab, added to paclitaxel, was shown to be beneficial in terms of prolonged progression free survival in a randomized phase III trial which included patients with ER- and PR-positive, as well as ER- and PR negative disease. The majority of patients in this study were HER2-negative and in the subset analysis ER-/PR-positive and negative patients had a similar benefit from bevacizumab. Our group demonstrated that basal-like and HER2 positive tumours have high angiogenic index, given the biological basis for this type of response. One of the promising new agents for the treatment of TNBC are poly(ADP-ribose) polymerase (PARP) inhibitors. A subset of PARPs is specifically involved in the detection of single strand breaks and the recruitment of base excision repair elements. In cells with alterations in BRCA function, as is often seen in TNBC, DNA repair processes are largely dependent on PARPs. Inhibition of PARP in these cells ultimately leads to cell death. Phase I data for the PARP inhibitor olaparib (AZD2281) suggest antitumor effectiveness in cancers associated with the BRCA1 or BRCA2 mutation. Preliminary results of a recent randomized phase II study with the PARP inhibitor BSI-201, combined with carboplatin and gemcitabine in metastatic TNBC, showed significantly improved clinical benefit rate, progression free survival and overall survival. However even the tumors with known targets might eventually gain resistance to treatment, or might not respond accordingly to the expected. There is a growing consensus that one way to approach this therapeutic issue is by addressing it from a more generic way. A possible solution is the inhibition of pathways common to tumor development that have a proven role in carcinogenesis. The PI3K is one of the most commonly altered pathways in human tumours. The pathway can be activated by receptor tyrosine kinases (RTKs) as well by genetic or epigenetic events of its key pathway components. The fast development of PI3K inhibitors either multi, pan or more recently isoform specific, has allowed not only a more broad understanding of this pathway but also provided feasible candidates for clinical evaluation either alone or in combination. The responsibility of selecting patients for therapy is challenging and appealing at the same time, but pathologists have to admit that the value of the specialty has increased since actively participating in the daily

workup of breast cancer patients. Nevertheless, the methodologies and interpretation expertise still need to be improved to assure the pathologists' place.

Suggested references:

Carvalho S, Schmitt F. Potential role of PI3K inhibitors in the treatment of breast cancer. Future Oncology 6: 1251-1263, 2010.

Katz M, Amit I, Citri A, Shay T, Carvalho S, Lavi S, Milanezi F, Lyass L, Amariglio N, Jacob-Hirsch J, Ben-Chetrit N, Tarcic G, Lindzen M, Avraham R, Liao YC, Trusk P, Lyass A, Rechavi G, Spector NL, Lao SH, Schmitt F, Bacus SS, Yarden Y. A reciprocal tensin3-cten switch mediates EGF-driven mammary cell migration. Nature Cell Biology 9: 961-969, 2007.

Milanezi F, Carvalho S, Schmitt FC. EGFR/HER2 in breast cancer: a biological approach for molecular diagnosis and therapy. Expert Review Molecular Diagnosis 8: 417-434, 2008.

Sousa B, Paredes J, Milanezi F, Lopes N, Martins D, Dufloth R, Vieira D, Albergaria A, Veronese L, Carneiro V, Carvalho S, Costa JL, Zeferino L, Schmitt F. P-Cadherin, vimentin and CK14 for identification of basal-like phenotype in breast carcinomas: an immunohistochemical study. Histol Histopathol 25: 963-974, 2010.

Podo F et al. Triple-negative breast cancer: Present challenges and new perspectives. Molecular Oncology 4: 209-229, 2010.

QUESTIONS

1. Which of the following profiling allow to classify a breast carcinoma as having a basal phenotype:

   a) ER negative, PR negative and HER2 negative.
   b) ER positive, HER2 negative and CK 5 positive.
   c) ER negative, HER2 negative and CK 5 positive. XXX
   d) ER negative, HER2 positive and P-cadherin positive.
   e) ER positive, HER2 positive and P63 positive.

2. Please mark the correct assertive:

   a) Molecular profiling is essential to sub-divide ER negative tumours according the prognosis.
   b) Breast carcinomas that co-express HER2 and EGFR have better response to trastuzumab.
   c) P-cadherin expressing is related to good prognosis in breast cancer.
   d) High Ki-67 is one of the markers of luminal A carcinomas.

e) Luminal phenotype is the most common in invasive breast carcinomas. XXX

3. Please mark the incorrect assertive:

f) ER luminal tumours have low proliferative index.
g) EGFR, HER2 and VEGF are potential targets in basal-like tumours. XXX
h) Trastuzumab and lapatinib are drugs used in HER-2 overexpressed breast carcinomas.
i) Oncotype Dx assay evaluate hormonal receptors, HER2 and proliferation.
j) CK14, vimentin and P-cadherin are markers that can identify basal-like tumours among triple-negative carcinomas.