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A bad tumor biomarker is as bad as a bad drug: The gap between genomics data and phenotype to predict response

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A B S T R A C T

The search for novel prognostic and predictive parameters in breast carcinoma is relentless. The new technological advances in gene expression profiling and in mutational analysis will hopefully prove to be clinically useful in informing the choice for the systemic therapy. For the time being, we are still relying in established immunohistochemical markers, namely estrogen and progesterone receptors, HER2 and Ki67. Advances in the harmonization of pre-analytical, analytical and interpretative variables may improve accuracy and reproducibility of the results.

Introduction

Certainly there are good and bad biomarkers of breast carcinoma, and we are continuously running experiments both in vitro and in vivo to try and identify novel and powerful biomarkers. However, despite all our efforts, only a few of them have proven to be clinically useful thus far. Expression of estrogen (and progesterone) receptors has limited prognostic value but unvaluable predictive value in the identification of patients candidate to endocrine therapy; HER2 overexpression and/or amplification has a remarkable prognostic value and at the same time identifies candidate patients to anti-HER2 treatment. Ki-67 is another widely used prognostic marker, despite difficulties in the harmonization of the analytical phase and of the interpretation of the results. Currently, these are the only available biomarkers in the daily practice.

Unfortunately, even when used in the best possible way, these biomarkers may only allow to identify candidate patients to a given treatment, but they are not able to predict who are the patients that will actually benefit from the treatment. As a result, we continue to offer endocrine therapy to patients with ER-positive breast cancer, or a targeted therapy to those with HER2-positive disease, knowing that only a (minor) fraction of them will actually benefit from these interventions – and we do not know how to identify them. The extensive search for positive biomarkers of response (or even for markers of resistance to the treatment) has largely been unfruitful. Certainly there have been signals stemming from recent studies of gene expression profiling and of mutational analysis that some molecular signatures or aberrations of specific genes might help identifying patients responsive or resistant to a given treatment, but none of these biomarkers has eventually entered the clinical practice. I am alluding, for example, to the gene signature predicting response to trastuzumab in the NSABP trial B-31 [1], or to the role of PIK3CA mutations in identifying patients resistant to this drug [2].

We are now entering the era of “precision medicine”, an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person (as per the definition of the National Institutes of Health in the United States). This is a terribly important initiative, but it may be particularly difficult to achieve precision medicine for patients with breast carcinoma. Except for the unfortunate women carrying germline mutations of a handful of genes, we do not know much about the etiology of breast cancer, and about any environmental or lifestyle-related agents that might correlate with an increased risk of developing the disease. If it is that difficult to prevent breast cancer, then we have to focus on early detection and better treatment to accomplish the goals of precision medicine.

Informing the systemic treatment of patients with ER-positive early breast cancer

The vast majority of breast cancer patients are diagnosed with an estrogen receptor (ER)-positive (and HER2-negative)

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disease, and are candidate to receive endocrine therapy with or without chemotherapy. We know this is a highly heterogeneous population of patients for the different histopathological and biological characteristics of their tumors, for the different risk of early or late relapse, and for the different response to systemic therapies.

Tailoring treatment for these patients requires to address a number of questions, starting with the identification of patients who would deserve addition of chemotherapy to endocrine therapy. The oncological community has long debated this issue, and has proposed a number of variables that could be helpful in informing the choice of the treatment. Tumor stage and grade, age and ethnicity of the patients are some of the parameters initially taken into account and still playing an important role in this setting. Focus on the biological characteristics of the tumors has opened new and promising perspectives: a better understanding of the clinical implications of hormone receptors and HER2 status, and of the proliferation markers like Ki67, has led to a "response-oriented" classification of breast carcinoma with the identification of highly endocrine responsive, partially responsive, and non endocrine responsive tumors [3]. Differential expression of hundreds of genes then allowed to re-classify breast cancers according to their unique expression profile in 4–6 major classes, including Luminal A, Luminal B, normal cell-like, HER2-enriched and basal-like [4]. This classification proved to have prognostic value, and it was adopted by the panelists of the St. Gallen Breast Cancer Conference in 2011 to inform the choice of different systemic treatments for each of these classes [5]. Taking into account the possible lack of resources to perform gene expression profiling assays in all the centers, it has been suggested to endorse immunohistochemical surrogates of these molecular classes for use in the clinical practice. Therefore, high accuracy and reproducibility of the pre-analytical and analytical phases of the immunohistochemical (and in situ hybridization) assays, and of interpretation and reporting of the results is absolutely needed. Huge international efforts have been devoted to ensure better harmonization of the assays for ER and progesterone receptors (PgR) [6], for HER2 [7] and for KI67 [8] testing in breast cancer.

A still debated question is whether or not there are definite thresholds for allocating ER-positive and HER2-negative tumors to the Luminal A or Luminal B class: at the same 2001 St. Gallen Conference it was proposed to use Ki67 labeling index as a reliable discriminator between these luminal classes adopting the 14% cut-off. This was derived from the pivotal study of Maggie Cheang and colleagues [9], showing that this was the cut-off resulting in the best correlation with the molecular classification of ER-positive breast cancer. The prevalence of Luminal A tumors in the Cheang's study was approximately 60% of the entire population of ER-positive cases. Surprisingly enough, when we tested the same 14% cut-off for Ki67 in a retrospective series of ER-positive breast cancers (n = 4747) diagnosed at IEO in 2008–2010, we found that the prevalence of Luminal A and Luminal B cases would be opposite to our expectations, with the former representing only approximately 32% and the latter 68% of the entire population. When we raised the cut-off to 20%, then the relative prevalence of the 2 tumor classes did approach the expected figures (i.e.: 55% Luminal A vs 45% Luminal B).

Likely, this was also the experience of others, because at 2013 St. Gallen Conference [10] the 14% cut-off for KI67 was challenged, and the majority of the panelists suggested to raise it to 20% for a better separation of luminal tumors. At the same time, however, according to the seminal article by Aleix Prat and colleagues [11], PgR expression was introduced as an additional parameter useful for a more accurate classification of these tumors. Accordingly, the immunohistochemical surrogates of Luminal A tumors would be ER positivity, HER2 negativity, KI67 labeling index lower that 20% and PgR higher than 20%.

Again, we had the opportunity of testing these new parameters in a large (n = 9415) series of patients with ER-positive and HER2 negative early breast cancer, treated between 1994 and 2006 and followed up at the European Institute of Oncology in Milan [12]. According to the 2011 St. Gallen criteria (Ki 67 cut-off of 14%), 33% of the tumors would have been classified as Luminal A, and 66% as Luminal B. Using the 2013 criteria (Ki67 at 20% and adding PgR with the 20% cut-off), 43% of the tumors would qualify for Luminal A and 57% for Luminal B.

More interestingly, the clinical outcome (distant disease-free interval) of the patients with low-proliferating tumors (i.e.: Ki67 < 14%) was not affected by PgR status (either less or more than 20%), as did for patients with highly proliferating (i.e.: Ki 67 > 20%) tumors. Conversely, patients with tumors showing an intermediate Ki67 labeling index (between 14% and 19%) had a significant different outcome according to PgR status. Accordingly, we proposed a new surrogate immunohistochemical definition for luminal tumors, suggesting to classify as Luminal A tumors with either low (<14%) Ki 67 labeling index, or with an intermediate labeling index (14%–19%) and PgR of >20%. Luminal B tumors would be defined by either high Ki67 labeling index (20% or more), or an intermediate KI67 and PgR ≤20%. Using this definition, 52% of the 9415 tumors would qualify for Luminal A, and 48% for Luminal B, with a significantly different clinical outcome of the patients (HR: 1.75, 95% CI: 1.46–2.11), after adjustment for clinico-pathological variables including for pT, pN, tumor grade, PVI, menopausal status and systemic therapy.

The recent technological advances and the extraordinary team efforts of consortia like the Cancer Genome Atlas (TCGA) [13] and the Molecular Taxonomy of Breast cancer International Consortium (METABRIC) [14] have dramatically increased our understanding of the molecular pathways and their derangements in human solid tumors. The combined evaluation of recurrent genomic abnormalities (gene mutations and gene copy number variations) and transcriptomic profiles has led to a continuous refinement of the molecular classification of breast cancer with prognostic implications. The original molecular classes have been further dissected in more and more subgroups, like the 10 integrative subgroups from the METABRIC consortium, or the complex arm-wise aberration index (CAAI)-positive and negative tumors [15]. Even within Luminal A breast cancers, commonly considered a relatively homogeneous subgroup of tumors with a good clinical outcome, a remarkable molecular diversity can be detected according to distinct gene copy number variations and mutation profiles [16]. The molecular stratification of Luminal A tumors allows to identify new subgroups with a significantly worse prognosis, and possibly to predict resistance to endocrine therapy.

Assessing responsiveness and resistance to HER2-targeted therapies

Mature survival data from the largest clinical trials of adjuvant trastuzumab have shown that the absolute benefit of the targeted therapy is in the order of 10% [17]. This has prompted a number of studies aimed at unveiling biomarkers predictive of responsiveness or resistance to trastuzumab and to other anti-HER2 agents.

The most obvious question has been whether immunohistochemical staining intensity and/or the number of HER2 gene copies could predict response to the treatment. Unfortunately, neither of these biomarkers could be correlated with response [18,19].

Possible mechanisms of resistance to trastuzumab "either de novo or acquired" have also been extensively investigated, based on data from in vitro experiments. Several putative biomarkers have

been tested in tumor samples of patients enrolled in clinical trials. Again, despite the expectations raised by the pre-clinical studies, the search for solid predictive biomarkers has been unsuccessful. Aberrations of c-Myc [20] and PTEN [21] did not correlate with response to trastuzumab in the N9831 trial of the North Central Cancer Treatment Group.

PIK3CA mutations did correlate with the likelihood of pathological complete response (pCR) in the neoadjuvant setting, where the rate of pCR in patients treated with trastuzumab, or combinations of trastuzumab and pertuzumab or lapatinib was lower in case of tumors harboring PIK3CA mutations. Interestingly, however, this did not translate in significant differences of long term outcome measures. When tested on samples from patients enrolled in clinical trials of adjuvant therapy, the mutational status of PIK3CA did not correlate with trastuzumab benefit [2].

Also, the predictive role of the truncated forms of the HER2 receptor (commonly referred to as p95) has not been convincingly demonstrated in the clinical setting until now [22]. Finally, there are discordant reports on the actual power of tumor-infiltrating lymphocytes (TILs) to predict response to trastuzumab [23]. An initial study of 209 patients with HER2-positive disease in the FinHER trial concluded that higher levels of stromal TILs are associated with increased trastuzumab benefit, but this finding was not confirmed by the analysis of a larger population of patients of the N9831 trial.

Epilogue

It remains to be assessed when and how much a more comprehensive understanding of the molecular heterogeneity of breast cancer will affect the process of clinical decision making in the daily practice. We are still awaiting results of ongoing clinical trials with gene expression-based prognostic classifiers to eventually implement them in the clinic. For now, available predictive models to inform the systemic treatment of individual patients are still limited to a few established biomarkers (hormone receptor and HER2 status, and markers of cell proliferation). As new therapeutic strategies are being evaluated in clinical studies, new predictive biomarkers are being sought. This is the case for example of the aberrations of the BRCA genes, or of the modulation of immune checkpoints.

Conflict of interest statement

None declared.

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