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Integrating molecular profiling, histological type and other variables: Defining the fingerprint of responsiveness to treatment

Giuseppe Viale*

Department of Pathology and Laboratory Medicine, European Institute of Oncology, University of Milan School of Medicine, Milan, Italy

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SUMMARY

The landscape of prognostication and prediction of responsiveness to systemic therapy for breast cancer patients has been recently enriched by the development of molecular assays, which enable to explore the whole universe of gene expression in the tumour cells and to unveil new prognostic and predictive markers. These molecular markers might well be used in combination with the established ones to address the many open questions that still pave the way to a truly personalized treatment. The actual clinical utility of the molecular assays is being tested in randomized clinical trials that require an unprecedented coordination of the activity of clinical investigators, pathologists and translational researchers worldwide.

Introduction

It is truly exciting to witness the tremendous advances in our understanding of the clinical and biological implications of breast cancer heterogeneity, and the continuous progresses in tailoring systemic treatments for the patients. We have already seen our efforts rewarded by the decline in the mortality for a disease whose incidence is still increasing worldwide.[1] Basic, translational and clinical researchers have started coordinating their efforts to unveil new biological features of breast cancer that might become either targets for novel specific drugs or new predictive parameters to better tailor existing therapies. New clinical questions are being addressed, with the aim of improving the selection of the candidate patients to tailored interventions and eventually identifying those who will actually respond to these therapies. It would be extremely important, for example, to learn how to predict which patients could be spared chemotherapy, which would benefit most from aromatase inhibitors or be responsive to anti-HER2 targeted therapies to better tailor systemic interventions.

A new generation of randomized clinical trials for pre-defined subpopulations of breast cancer patients selected according to the biological characteristics of their tumours are being conducted and newly launched to prove the efficacy of tailored treatments. The design and the conduct of these new clinical trials require an unprecedented coordination of the activity of clinical investigators, pathologists and translational researchers worldwide.

* Correspondence: Prof. Giuseppe Viale. Department of Pathology and Laboratory Medicine, European Institute of Oncology, Via Ripamonti 435, 20142 Milan, Italy.
Tel.: +39 0257489419; fax: +39 0257489417.
E-mail address: giuseppe.viale@ieo.it (G. Viale).

The unexhausted search for new prognostic and predictive parameters

The advent of molecular assays exploiting the whole universe of gene expression in the tumour cells has enabled to identify new prognostic and predictive signatures whose actual clinical implications are currently being tested in randomized clinical trials.[2] These assays have brought into the scientific arena a completely new methodological approach to the search for new prognostic and predictive parameters. Indeed, the traditional approach aimed at assessing the clinical implications of the expression of individual candidate markers, selected by the researchers according to the indications stemming from pre-clinical investigations. Thus, scientists have looked at the clinical implications of p53, p21, p27, BCL2, and several other proteins, mostly performing retrospective studies on heterogeneous populations of patients. The actual power of these investigations to identify reliable and clinically useful markers was very limited, and they did not reach, which very few exceptions, any level of evidence high enough to be transferred to the clinical practice. Only a few markers have attained a higher level of validation in prospective randomized clinical trials. As a result, despite the huge efforts and amount of resources spent in the last decades, none (with the exception of uPA/PAI-1) of the recently investigated markers has been ranked among those recommended for clinical use by the ASCO panellists of the 2007 recommendations for the use of tumour markers in breast cancer.[3]

Looking *a posteriori*, the failure of the traditional approach to search for new clinically useful markers could have been largely expected. Indeed, we now know that the biological pathways controlling tumour cell proliferation and differentiation, and the response to different therapeutic strategies are highly redundant, and that the same molecules may be involved in different

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pathways, sometimes exerting opposed effects under different stimulations. It is not conceivable that looking for the expression of just one or a few molecules would allow to conclusively ascertain tumour behaviour. Also, many if not all the markers investigated in the past are tightly correlated with several other proteins in the intracellular networking of the different biological pathways, so that in several instances the apparent clinical value of a given parameter was not retained when multivariable analyses including already known parameters have been conducted. Finally, the available investigational techniques – and especially immunohistochemistry – have also important limitations, mainly due to the fact that they cannot be used for high throughput studies looking at multiple markers simultaneously in a large series of cases, and that they could not provide precise quantitative results.

It is now clear that neoplastic transformation, tumour progression and response to treatment are driven and accompanied by the deregulated expression of hundred or thousand genes, whose status cannot be assessed by the traditional approach. The novel molecular techniques of gene expression profiling allow to evaluate the expression of thousand of genes in a large number of tumours, and compare the expression profile of individual tumours with that of the normal parent cells, or of other similar tumours. This approach may highlight which genes are involved in the neoplastic transformation, and which are determining tumour progression or responsiveness to the therapy. This is a new philosophy inspiring the search for new prognostic and predictive parameters. It is no longer the researcher who selects a marker which might be worth investigating, but the tumour cells themselves are asked to reveal what is a truly informative assembly of parameters to be assessed. Also, there are no limitations to the number of different genes which can be evaluated with these techniques, so that a fully comprehensive portrait or profile of the deranged molecular pathways can be obtained. From the tremendous amount of data reflecting the expression of thousand of genes, a minimum set of genes whose expression is hierarchically dominating the different aspects of the cellular behaviour has to be derived. This may eventually lead to the identification of a finite number of informative parameters, whose clinical usefulness has to be validated before they are applied in the daily practice. Once a minimum number of informative parameters to answer specific clinical questions has been identified, then the most appropriate technical approach for their application in the clinic could be selected, be it gene expression profiling, RT-PCR or even "targeted" immunohistochemistry for the relevant gene products.

Molecular assays have elicited a great deal of expectations, and for the most part they have been enthusiastically welcomed as potentially offering new chances for a better and more personalized care of the patients. Many, however, are still reluctant to consider these assays ready for use in the clinical practice, and keep waiting for a confirmatory evidence of their utility when the results of ongoing clinical trials will be mature. Finally, the scientific debate is also enriched by the contributions of scientists and clinicians maintaining a more sceptical view about the clinical utility of molecular assays, that they consider only a more expensive and sophisticated way of pursuing the same information already accessible by the current clinico-pathological evaluation.

Assessing the risk of tumour progression

Traditionally, patients are allocated into different risk categories based on both the clinical characteristics and the pathological and biological features of their primary tumour. Besides the age of the patients, the established prognostic parameters are tumour type, size and grade, the proliferative fraction, the occurrence of peritumoral vascular invasion, the hormone receptor and HER2/neu status, and the status of the regional lymph nodes.[4] More recently, gene expression profiling experiments have revealed different signatures for tumours with high and low risk of progression, according to the differential expression of a finite number of genes.[5,6] The prognostic value of the expression signatures has been compared favourably with some of the traditional clinico-pathological assessments of the risk of recurrence, as derived by the St. Gallen recommendations, the Nottingham scoring system and Adjuvant! Online.[7] These signatures have been validated in independent cohorts of patients with both node negative and node positive disease, and one of them (MammaPrint™, Agendia BV, Amsterdam, The Netherlands) has obtained FDA clearing and it is now commercially available. Another commercially-available assay (Onco*type* DX™, Genomic Health, Inc., Redwood City, CA)[8] has been considered by the ASCO panellists of the 2007 recommendations for the use of tumour markers in breast cancer[3] and by the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (www.ncnn.org) useful to predict the risk of recurrence in patients with node-negative and oestrogen receptor positive disease treated with tamoxifen. The panellists cautioned however that it is not known whether these conclusions generalize to hormonal therapies other than tamoxifen[3] and that the assay should be used for decision making only in the context of other elements of risk stratification for individual patients (NCCN). The members of the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group found no direct evidence regarding the clinical utility of the MammaPrint™ assay, and inadequate evidence regarding Onco*type* DX™[9] to improve outcomes in patients with breast cancer. They found, however, encouraging indirect evidence for Onco*type* DX™ and plausibility for potential use of MammaPrint™.[9]

Predicting the responsiveness to treatment

It is now widely accepted that the "one fits all" therapeutic approach to patients with breast carcinoma is not longer justified, and all the efforts are devoted to better tailor systemic therapies for individual patients. Fortunately, the range of possible therapeutic options is continuously increasing, and the treating physicians may now select the more appropriate treatment among several different agents with endocrine or cytotoxic effects, used alone or in combination, with the possible addition of targeted anti-HER2 interventions.

According to the 2007 St. Gallen Consensus,[4] endocrine responsiveness may be predicted by the combined evaluation of oestrogen (ER) and progesterone (PgR) receptors, HER2 and Ki-67. Tumours are considered to be highly endocrine responsive if they express ER and PgR in the majority of the cells, do not show amplification or over-expression of the HER2 gene, and have a low proliferative fraction. Tumours with an incomplete endocrine responsiveness are characterized by a low expression of ER and PgR, or lack of PgR (irrespective of the ER status) or overexpression/amplification of the HER2 gene, or high proliferative fraction. Finally non endocrine responsive tumours may be identified by the lack of any immunoreactivity for ER and PgR, and these tumours may be exquisitely sensitive to cytotoxic chemotherapy.

Based on the assessment of hormone receptor and HER2 status it should be easily feasible to identify candidate patients to endocrine or cytotoxic treatments and to anti-HER2 interventions. Unfortunately, the false-negative and false-positive rates in the assessment of these biological parameters still is unacceptably high, as it is the intra- and interlaboratory discordance rate. It has been repeatedly shown that the false-negative rate for ER and PgR may be as high as 20%, whereas the false-positive rate is 2–4% for ER but nowadays much higher (up to 15%) for PgR. The current higher rate of false-positive results with PgR assays is likely related to the use

of the newly developed monoclonal antibodies raised in rabbits.[10] An inaccurate assessment of hormone receptor status has dramatic implications for the patients, who can be denied a potentially useful endocrine therapy in case of a false-negative result, or may be offered an ineffective and potentially harmful treatment because of a false-positive assay.

Also, the HER2 negative status should be carefully assessed not to miss candidate patients to anti-HER2 tailored therapies. Luckily, the definition of a HER2 negative status is not controversial, and it is based on the lack of over-expression of the protein (as evaluated by immunohistochemistry) and on the lack of gene amplification (as documented by in situ hybridisation techniques, with either fluorescent [FISH] or chromogenic [CISH or SISH] probes). Again, as for the evaluation of the hormone receptor status, the need for an accurate assessment of HER2 status cannot be over-emphasized. Unfortunately, despite the availability of standardized reagents and protocols for both immunohistochemical and in situ hybridization assays, and the publication of several guidelines and recommendations for an optimal testing, the assessment of HER2 status is still plagued by a high rate of false-positive and false-negative results.[11]

All the pathologists involved in the care of breast cancer patients should become more and more aware of the tremendous clinical implications of the assessment of hormone receptor and HER2 status of breast cancer. Too often the "expert" pathologists devote all their efforts to the "noble" task of reaching the correct histopathological diagnosis of breast cancer, and the more challenging it is, the more excited they are. When it comes to the assessment of the receptor status, they feel much less interested, and they happily leave this task to less experienced colleagues. Too often it is intolerably tedious to critically evaluate the immunostaining results, to update the staining protocols, to check for the consistency of the results over time, or to supervise the reports of internal and external quality controls. Too often there is not enough time to attend the multidisciplinary sessions, and the opportunity of discussing the clinical implications of the pathological report with the treating oncologists is therefore missed. This is no longer acceptable when considering the preeminent role of the accurate assessment of the biological features of breast cancer for a proper tailoring of the systemic therapy.

As for prognostication, the new molecular assays may also be of assistance in predicting the responsiveness of the tumours to different therapeutic approaches. Different gene expression profiles correlated with the likelihood of complete pathological response to neoadjuvant chemotherapy, and Onco*type* DX™ predicted benefit from Tamoxifen in patients with a low to moderate recurrence score (RS), and benefit from chemotherapy (particularly CMF) in those with a high RS.[12] More recently, an expression profile of 205 covariables has been shown to discriminate between tumours clinically responsive or resistant to Letrozole after 3 months of neoadjuvant treatment.[13]

To replace or to complement the traditional biological parameters?

It may become more and more difficult, with the increasing amount of available data on the clinical utility of different assays, to decide which is best suitable to accurately stratify the patients according to their risk, and to predict the response to the different treatments. The attitude to immediately replace any established assay and parameter with those emerging is seductive but it is oversimplistic and may not be truly rewarding.[14] This is best exemplified by the increasing adoption of the molecular classification (luminal types, HER2 positive, basal-like) of breast cancer[15] by clinicians and pathologists to replace both the traditional histopathological classification and the one based on the endocrine responsiveness of the tumour. This may be highly misleading, because the molecular classes are heterogeneous and encompass different tumour types with different risk profiles and different responsiveness to the therapy.[16] Not all the tumours with the basal-like profile are high risk tumours with an ominous prognosis, because this molecular class also includes low-grade metaplastic carcinomas (fibromatosis-like carcinomas and low-grade adenosquamous carcinomas), adenoid-cystic carcinomas, medullary carcinomas and low-grade apocrine carcinomas, which have a very favourable prognosis. Furthermore, that all basal-like carcinomas do not benefit from targeted endocrine or anti-HER2 interventions is also incorrect, because at least 20% of these tumours do express ER and/or overexpress HER2.[17] In addition, a simple immunopanel of ER, PgR, HER2 and Ki-67 can serve as a surrogate for much of the clinically relevant information generated by the molecular classification, and it has been shown to have strong prognostic value and to predict the benefit of the addition of taxanes to tamoxifen.[18]

That the response of the tumours to neoadjuvant chemotherapy may be predicted by molecular profiling is undoubtedly true,[19–21] although in one of these studies it has also been stated that these assays do not provide additional information when tumour grade, hormone receptors and HER2 status are known.[20] The recurrence score derived by Onco*type* DX™ is also significantly correlated with histological (nuclear grade, tubule formation, mitotic index) and biological (ER, PgR and HER2 status) characteristics, and it may now be predicted by the combined evaluation of these parameters.[22]

If all the molecular assays were just a more sophisticated alternative to the assessment of established markers, aimed at overcoming the lack of accuracy and of reproducibility of the conventional assays, it would be very disappointing. It would be much wiser to invest time and resources in fostering awareness and expertise among the pathologists, than to design and conduct randomized clinical trials to assess the actual clinical utility of the molecular assays in refining the risk allocation of the patients and in better tailoring the systemic therapies for a more personalized treatment.

The substantial promise of the molecular assays is that they can provide additional prognostic and predictive information beyond what is currently available using the established assays. The ongoing randomized clinical trials will eventually confirm or disprove the clinical utility of the MammaPrint™ signature (evaluated in the MINDACT trial) and of Onco*type* DX™ (in the TAILORx trial) to better identify the patients needing chemotherapy, and to avoid the risk of a suboptimal treatment.

Integrating molecular profiles and pathological variables

While waiting for the results of the randomized clinical trials and of additional studies addressing the question of the clinical utility of the molecular assays, it may be expected that an integrated approach to prognostication and prediction of response to therapeutic agents will eventually be of the greatest benefit for breast cancer patients. We will have to learn how to address different questions by using the most cost-effective assay in a hierarchical manner, starting from the basic clinical and pathological data, and adding stepwise the relevant immunophenotypic and molecular profiles, as they are needed and available. This combined approach requires the coordinated and careful efforts of clinicians, pathologists and molecular biologists, to assure that the entire spectrum of the relevant data are accurately and reproducibly derived from each of the subsequent analyses.

There are instances where morphology alone allows to collect all the currently available and relevant prognostic and predictive parameters, thus portraying the tumour to our best potential

without the need for additional assays. This would be the case, for instance, of a high-grade invasive ductal carcinoma with central necrosis or fibrosis, whose accurate histopathological identification *per se* implies the highest risk of distant recurrence (particularly in visceral organs and brain) in a relatively short time after the diagnosis, and a reduced length of survival following the first distant relapse, despite exquisite responsiveness to cytotoxic chemotherapy.[23,24] The prognostic and predictive value of the pure morphological identification of these tumours is almost unaffected by any additional information, be it the size of the tumour, the number of affected lymph nodes, the triple-negative phenotype as assessed by immunohistochemistry, or the basal-like profile as derived by the molecular assays.

It would be truly unwise to underestimate the possible contribution of the accurate histopathological assessment of tumour type and grade in the evaluation of the prognostic and predictive profile of breast cancer. Certainly, the fraction of tumours whose peculiar morphological features offer the whole set of the currently available prognostic and predictive markers is very small, and mainly includes the special types of breast cancer with a very favourable prognosis when detected at an early stage of the disease, as the pure cribriform and pure tubular carcinomas, the adenoid-cystic carcinomas, and the classic variant of lobular carcinomas. For the vast majority of breast cancers, a more complex assembly of variables has to be sought, in addition to the morphological features. This primarily includes the quantitative evaluation of hormone receptor status (scored as the percentage of immunoreactive cells alone or in combination with the staining intensity) and the assessment of HER2 gene amplification and/or overexpression to guide the treating physician in the choice of the most appropriate systemic treatment. The evaluation of the tumour proliferative fraction by the Ki-67 labelling index may provide additional prognostic information and help in finely tuning the therapeutic plan.[25,26] The resulting prognostic and predictive portrait, however, in the majority of the cases is still far from allowing the stratification of the risk at the individual level or a truly personalized systemic treatment. Several questions remain to be addressed before we succeed in identifying the responsive patients among those who are candidate to a given treatment. This would avoid the administration of potentially harmful agents to patients unlikely to benefit, without denying a potentially useful treatment to those patients likely to respond. Here is where molecular profiling might be of the greatest assistance, because the traditional assays have until now failed to identify all the patients who could be spared chemotherapy, or who are getting the greatest benefit from the treatment with aromatase inhibitors or with anti- HER2 interventions or with taxanes.

There is room for being confident that these and other questions will be more successfully addressed by the integration of the established assays with the new ones. It has been recently shown that within apparently homogeneous cohort of tumours according to hormone receptor and HER2 status, gene expression profiling identifies subsets of tumours with significantly different relapse-free survival, and that the models for predicting the risk of relapse in node-negative breast cancer based on the combination of both molecular data and clinicopathological variables are more robust than those construed with either set of data alone.[27] Certainly, before the new molecular assays could be widely adopted in the daily practice once they will be clinically validated, several other issues remain to be addressed, including their feasibility and reproducibility in laboratories other then the current centralized testing facilities, and their suitability for formalin-fixed and paraffin-embedded tissue samples (MammaPrint™ still requires the availability of frozen tissue samples). More important, however, is the continuous need for the accurate judgement and the open-minded attitude of all the scientists and clinicians actively pursuing the best chance of care for breast cancer patients.

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