Gene Profiling Assay and Application: The Predictive Role in Primary Therapy

Debora Fumagalli, Christine Desmedt, Michail Ignatiadis, Sherene Loi, Martine Piccart, Christos Sotiriou

Correspondence to: Christos Sotiriou, MD, PhD, Breast Cancer Translational Research Laboratory, Faculty of Medicine, Université Libre de Bruxelles, Institut Jules Bordet, 125 Blvd de Waterloo, Bruxelles 1000, Belgium (e-mail: christos.sotiriou@bordet.be).

Several treatment options, including endocrine therapy, chemotherapy, and targeted therapy, have been shown to improve survival of breast cancer patients. Currently, clinical tests for predicting cancer response are not available, and individual markers have shown little predictive value. Several gene expression profiling studies have been carried out in the attempt to identify predictive signatures. The neoadjuvant setting revealed to be ideal for this purpose because it allows the direct assessment of response to treatment, and tumor is readily available for multiple time point biopsies. Although the results are promising, at the moment, none of these signatures has been proven to be of sufficient discriminatory power to be used in clinical setting. More effective therapies targeted to specific subsets of patients, accurate and standardized definition of therapeutic response, and properly designed clinical trials are required before microarrays can reliably be used as tools for clinical decision making.


Microarray technology has been used in breast cancer in the attempt to define gene expression signatures able to predict the response to treatment of patients enrolled in clinical trials (1–11). To this end, the neoadjuvant setting has shown to be the most suitable because it allows the direct assessment of response to chemotherapy by monitoring changes in tumor size during treatment; the tumor is readily accessible and it allows for serial biopsies; pathological complete response (pCR) after treatment has a potential as surrogate marker for long-term breast cancer outcome (12).

Predictors of Response to Chemotherapy

Several chemotherapy regimens are now used in primary treatment of breast cancer. The first attempts to define molecular predictors of response have identified cancer features or molecular markers that predict generic chemosensitivity rather than predicting sensitivity to a specific chemotherapy regimen over another.

It is now recognized that some clinical features, such as negativity for estrogen receptor (ER), positivity for human epidermal growth factor receptor 2 (HER2), high proliferative activity, and high histological grade are associated with chemosensitivity in breast cancer. In recent years, investigators attempted to test the ability of clinically available prognostic multigene assays (13–15) to predict chemotherapy benefit, both in the adjuvant (16–18) and in the neoadjuvant settings (19–21). These retrospective investigations showed that multigene assays such as the Oncotype DX (13), which first entered the clinical practice as prognostic tool for women with node-negative, ER-positive early-stage breast cancers, are able to assign these patients to diverse risk categories that benefit differently from chemotherapy. Patients considered to have a low risk of recurrence according to Oncotype DX are the one gaining less benefit from the use of chemotherapy; vice versa, patients considered to be at high risk of recurrence benefit more from chemotherapy treatment. However, it has been pointed out that the predictive component of these so-defined “first generation prognostic signatures” relies on their ability to measure proliferation, and they are limited to convey information on “generic” chemosensitivity (22).

A series of studies has then tried to predict the sensitivity to specific drugs or regimens. For instance, Hess et al. (3) evaluated gene expression profiling as a potential toll to predict pCR to sequential paclitaxel-anthracycline preoperative chemotherapy. Diverse predictors of pCR were developed from 82 patients, and their accuracy was assessed on 51 independent patients with stage I–III breast cancers treated with weekly paclitaxel and fluorouracil–doxorubicin–cyclophosphamide (T/FAC) chemotherapy. Among several identified predictors that performed equally well, a 30-probe set diagonal linear discriminant analysis (DLDA-30) classifier was selected for independent validation and it showed significantly higher sensitivity (92% vs 61%) than a clinical predictor including age, grade, and ER status. In a recent publication by the same group (7), the performance of DLDA-30 was evaluated in a prospective, randomized neoadjuvant clinical trial comparing T/FAC vs FACx6. Even if the assay was predictive of response to T/FAC chemotherapy with an apparent regimen specificity, in this trial its performance was similar to that of the same multivariate clinical prediction model tested in the first work. This probably indicates that DLDA-30, as other genomic predictors developed with a similar strategy, catches mostly gene expression information associated with clinical phenotype and that new approaches are needed to develop clinically useful genomic predictive tools.

In the recent past, our group led a prospective neoadjuvant clinical trial in which ER-negative breast cancer patients were treated with anthracycline monotherapy with the specific aim to evaluate the predictive value of topoisomerase IIα and to develop a gene expression signature to identify patients who do not benefit from anthracyclines (8). An “anthracycline-based score (A-Score)”
was developed that combines three different signatures associated with the efficacy of anthracyclines (a topoisomerase IIa signature and a stroma and immune response signatures, previously published [23]) and that turned out to have a high negative-predictive value both in the overall population and in the two subgroups of HER2-positive and HER2-negative patients. This is to indicate that studies on targeted populations that explore the predictive role of tumor microenvironment (6) or pathway activation (9) are with all probability an effective way to move forward.

**Predictors of Response to Endocrine Therapy**

Several randomized trials have assessed the value of neoadjuvant endocrine therapy in postmenopausal women with ER-positive breast cancers, with either tamoxifen, aromatase inhibitors, or their combination (24,25), and an attempt to obtain predictive gene expression signatures has been done also in this setting (10,11).

As an example, we report on the study in which Mello-Grand et al. (11) tried to identify an expression signature predictive of response to neoadjuvant treatment with anastrozole in patients with ER-positive breast cancers and, at the same time, to delineate treatment effects and possible mechanisms of intrinsic resistance occurring in non-responder patients. The authors analyzed the transcriptome of 17 tru-cut biopsies before treatment and 13 matched surgical samples after 3 months treatment with anastrozole, and they correlated molecular profiles to clinical response. Interestingly, the authors observed that treatment with anastrozole was associated with a decreased expression of genes involved in cell proliferation and an increased expression of genes involved in inflammatory processes, showing that these studies could provide mechanistic insights into the processes of drug sensitivity or resistance.

**Predictors of Response to Targeted Therapy**

A constantly increasing number of targeted drugs are tested in clinical trials enrolling breast cancer patients, and investigators are eager to identify predictive markers able to select patients most likely to respond.

We report on a recent work (26), in which the investigators attempted to identify potential molecular predictors of response to preoperative single-agent everolimus, a rapamycin derivative that acts as an inhibitor of mammalian target of rapamycin (mTOR) a serine/threonine kinase that plays a key role in the cancer-related pathway PI3K/Akt/mTOR. Core biopsies were taken before and after treatment in 27 ER-positive patients who completed 11–14 days of treatment. Changes in proliferation (Ki67) and phospho-AKT were measured by immunohistochemistry on diagnostic core biopsies/resection samples embedded in paraffin to determine response to treatment, and RNA extracted from frozen core biopsies/resection samples underwent gene expression profiling. Analysis of pretreatment samples suggested that there were two distinct groups of tumors, which responded to everolimus, and one group that did not, each with different expression profiling. The comparison of pre- and posttreatment gene expression profiles was also able to detect changes in genes and pathways in both the all population and in responders vs nonresponders.

Even if there are limitations in this study, such as the small number of patients, its feasibility shows that the preoperative setting could be used to investigate the effects of targeted agents. In this regard, the so-called window-of-opportunity trials, in which a new drug is administered as single agent in treatment-naïve patients for a short course before surgery, hold promise for the investigation of the mechanisms of action of new drugs.

**Alternative Strategies to Define Multigene Predictors**

An alternative strategy that has been used to generate predictive multigene assays is the in vitro signature analysis. Usually, in this approach, gene expression data and in vitro drug response information from cell line panels are used to generate drug-specific pharmacogenomic response predictors that can be applied to human data (27). Unfortunately, several investigators failed to reproduce in human cases the discriminating power of purely cell line–derived drug-specific predictors (28–30), highlighting the difficulties of cell line models to capture patient-related differences in drug metabolism, and the influence of tumor microenvironment in response to treatment.

**Unresolved Issues and Future Perspectives**

Despite the promises, none of the signatures generated so far has been approved for use in clinical setting. Different issues could be implicated in their failure.

1. The statistical confidence in the results of most of the studies conducted so far is limited because of the small sample sizes and multiple comparisons (31). Studies with a larger number of subjects and with an appropriate design should provide more clinically useful results. The ideal setting to develop treatment-specific predictors would be a randomized trial properly powered to identify individual genes with significant treatment–marker interaction effect; however, the planning of such a trial is not free from statistical and biological difficulties.

2. Different studies used different parameters of tumor response; moreover, at the moment the definition of a fundamental endpoint such as pCR is not standardized, causing substantial confusion in the interpretation and comparison of results generated by neoadjuvant trials. A stricter uniform definition of pCR and other endpoints is required to reduce subjectivity and strengthen its role in predicting overall survival in the context of both developing new therapies and determining molecular signatures.

3. Most of the available studies were carried out in unselected breast cancer populations. Several studies have shown that breast tumors can be grouped accordingly to at least four different subtypes with distinct clinical outcomes and response to treatment, namely the triple-negative, the HER2-like, and at least two luminal-like subtypes, luminal A and B (32–37). If different molecular classes have different sensitivity to chemotherapy, using data from all cases will likely yield predictors that primarily discriminate between molecular classes and have less strength to predict response within a class, and the predictive value of a biomarker that is specific for a single subtype will be diluted by the presence of other subtypes.
We strongly believe that the use of more effective therapies targeted to specific subsets of patients, the definition of more accurate endpoints, and the design of smarter clinical trials intended to get insights into the mechanistic effects of drugs could provide a great contribution to the development of a new class of predictive signatures with clinical impact (Figure 1).

References


Affiliation of authors: Breast Cancer Translational Research Laboratory, Faculty of Medicine, Université Libre de Bruxelles Brussels (DF, CD, MI, SL, CS), and Breast International Group (MP), Institut Jules Bordet, Brussels, Belgium.