Desmedt and colleagues published two articles, one in the June 1, 2007 issue, and the other in the August 15, 2008, issue of Clinical Cancer Research, that showed gene-expression signatures to be proliferation driven and time dependent, with their prognostic power decreasing with increasing follow-up years. Moreover, the articles showed that immune response is a crucial determinant of prognosis in the HER2-positive and estrogen receptor–negative/HER2-negative subtypes, providing a rationale to further explore the role of the antitumor immune response in these breast cancer subtypes. Clin Cancer Res; 21(21): 4743–6. ©2015 AACR.


Introduction
Breast cancer is the most common cancer in women and is still the most common cause of cancer-related death in women worldwide, accounting for more than 500,000 deaths annually (1). During the past two decades, intensification of adjuvant therapy in early breast cancer has allowed for a reduction of cancer relapse and improved mortality. However, almost two thirds of the patients that will receive such adjuvant therapy would have survived without it. One of the greatest challenges of modern oncology is identification of the patients that would truly benefit from an aggressive therapeutic approach, in order to avoid unnecessary toxicities in those that could be spared adjuvant chemotherapy. To do so, various clinical and pathologic factors such as age, tumor size, lymph node involvement, histologic grade, ER and HER2 expression status have been used as prognostic and predictive indicators for treatment-related decisions.

The development of DNA microarray technologies has given cancer researchers the unique opportunity to interrogate cancer cell biology by analyzing numerous tumor gene expression profiles (2). Comprehensive analysis of breast tumor gene expression revealed the transcriptomic heterogeneity of the disease and led to the classification of breast cancer into distinct molecular subtypes defined primarily by ER and HER2 expression status (3). The increasing access to these technologies and the availability of retrospective cohorts in institutional biobanks also triggered attempts to develop new prognostic models based on gene-expression profiles. While these gene-expression signatures are providing clinicians with significant information on tumor biology and aggressiveness, work still needs to be done to further unravel the true biologic meaning and clinical utility of such testing.

Proliferation-Based Prognostic Gene-Expression Signatures
Several multigene signatures were developed in the early 2000s (MammaPrint, 76–gene signature, Oncotype DX) by searching for tumor gene-expression patterns associated with clinical outcomes (4–6). To generate these signatures, genes for which expression was associated with clinical outcome were selected without a priori biologic assumption. The gene signatures were shown to have high sensitivity and better specificity than traditional clinicopathologic criteria to identify early breast cancer patients at high risk of mortality and relapse, therefore succeeding in identifying more low-risk patients that may not need adjuvant chemotherapy (4).

Interestingly, although the various gene signatures share similar prognostic value, there is little overlap between the genes selected (7, 8). Differences in the methods used to develop the signatures, dissimilarities in patient characteristics, and limited sample sizes may explain the discrepancies between the different signatures (9). Recently, disagreement between the different tests in assigning individual tumors into risk categories was demonstrated, with 52% of tumors assigned to different risk categories by different tests, thus raising questions about the accuracy of these tests at the individual level (10).

Concurrently, other gene-expression signatures were developed by interrogating genes associated with a specific biologic process such as histologic grade, wound healing, or invasiveness. These gene-expression signatures also succeeded in demonstrating additional prognostic value to clinicopathologic criteria (9, 11, 12). Interestingly, when comparing the prognostic performance of...
the 70-gene signature (MammaPrint) and a 97-gene signature developed to discriminate high-grade and low-grade tumor (Genomic Grade Index), similar separation in metastasis-free survival between low- and high-risk groups was reported, thus suggesting for the first time that proliferation may be the driving force behind the 70-gene and other gene signatures (8). Although cumulative evidence indicates that gene signatures can improve our prognostic evaluation of patients with early breast cancer, clinicopathologic criteria such as lymph node involvement and tumor size are still adding independent prognostic information to the gene signatures, as was demonstrated in a meta-analysis of 2,833 breast tumor gene expression profiles evaluating the prognostic ability of several gene-expression signatures (13).

Beyond Prognostication: Deciphering Key Biologic Processes in Breast Cancer

In 2008, our group reported the results of a comprehensive meta-analysis of gene expression and clinicopathologic profiles of 2,100 breast cancer patients whose gene expression and clinical data were available from public databases in a pivotal article published in this journal (14). We first defined seven in silico gene expression modules in an attempt to recapitulate key biologic processes in cancer, namely tumor invasion/metastasis, impairment of immune response, sustained angiogenesis, evasion of apoptosis, self-sufficiency in growth signals, and ER and HER2 signaling. The different gene modules were defined by selecting genes specifically coexpressed with the prototypic gene representing the particular process. Each of the 2,100 tumors was then classified in one molecular subgroup using the maximum probability of membership in the cluster. Tumors were also classified into breast cancer subtypes according to ER and HER2 gene expression (ER+/HER2−, HER2+, ER+/HER2+).

When analyzing biologic processes in the different breast cancer subtypes, we found that proliferation and histologic grade were the variables with the highest prognostic impact in the ER+/HER2− population, as could be expected from our previous data (11). However, in the ER+/HER2− subpopulation, only the immune response was significantly associated with relapse-free survival (RFS). In HER2+ tumors, tumor invasion and immune response showed significant correlation with clinical outcomes. Our study was the first to demonstrate that the prognostic significance of different biologic processes involved in carcinogenesis varies according to the breast cancer molecular subtype in which it is evaluated.

We also confirmed that proliferation-associated genes represent more than half of the genes included in the first prognostic gene-expression signatures. We underwent verification of the performance of different genetic tests (MammaPrint, 76-gene signature, p53 signature, Wound Healing signature, Genomic Grade Index, Oncotype DX, Invasiveness signature) to predict relapse in different breast cancer subtypes. All the signatures demonstrated good capacity to discriminate between high- and low-risk patients with ER+/HER2− breast cancer, but were much less informative in the other subtypes.

Our study underscored that breast cancer subtypes [ER+/HER2+, HER2+, ER+/HER2+] often referred to as triple-negative breast cancer (TNBC)] represent distinct biologic entities, and should be considered as such in the approach to biomarker and treatment development in breast cancer. Moreover, we identified crucial processes specifically associated with prognosis in ER+/HER2+ and HER2+ breast cancer, uncovering new potential therapeutic targets. For example, we confirmed the previous hypothesis raised by Teschendorff and colleagues that expression of immune-related genes is associated with improved clinical outcomes in ER+ breast cancer (15).

Since that time, the body of evidence demonstrating the influence of the immune response on HER2+ and TNBC evolution has enlarged tremendously. In a pooled transcriptome analysis of 996 breast tumors all treated with anthracycline-based or anthracycline and taxane-based neoadjuvant chemotherapy, high scores of immune gene-expression signatures were associated with an increased rate of pathologic complete response (pCR). Although the association was seen across all breast cancer subtypes, it remained statistically significant after multivariate analysis only in HER2+ and ER+/HER2+ tumors (16). Increased expression of immune-related genes was also associated with increased RFS in a transcriptome analysis of 1,282 HER2+ tumors from the NCT01785046 adjuvant trastuzumab trial (17). Moreover, the results of this study suggested that the benefit from trastuzumab was limited to those tumors with immune-related gene expression enrichment. In TNBC, analysis of gene expression profile of 587 tumors demonstrated gene clustering in six distinct molecular subgroups, among which was an immunomodulatory subtype associated with a more favorable prognosis (18).

Consistent with these findings, correlation between increased levels of tumor-infiltrating lymphocytes (TIL), as measured by pathologists, and improved pCR rate and clinical outcomes has been demonstrated in a large number of studies, with the most robust evidence being observed in the ER− population (19–21). Finally, a recent article has further shed light on how these lymphocytes in some tumors with extensive lymphocytic infiltration are organized in tertiary lymphoid structure participating potentially in a sustained antitumor immune response (22). Together, these data suggest that manipulating the immune system (priming immune response and/or releasing the break) could be a promising therapeutic option in HER2+ and TNBC, and phase I and II trials with immune checkpoint inhibitors are currently ongoing (NCT00083278, NCT0152591, NCT02129556).

Proliferation-Based Gene-Expression Signatures to Predict Early and Late Relapses

Following the demonstration of the prognostic value of one of the first reported gene signatures, namely the 76-gene signature, our group also endeavored to validate this signature in an independent cohort of 198 node-negative early breast cancer patients recruited via the TRANSBIG network in an article published in this journal in 2007 (23). The patients in our cohort had T1-2N0 breast cancer, and 134 had ER− tumors; the median follow-up was 14 years. In agreement with previous results (5, 24), we confirmed the capacity of the 76-gene signature to discriminate patients at low and high-risk of distant metastasis and survival, and to identify more low-risk patients compared with standard clinicopathologic criteria. The 5- and 10-year time to distant metastasis rates (TDM) were 98% and 94% in the low-risk group compared with 76% and 73% in the high-risk group, with a hazard ratio (HR) of 5.78.

Importantly, our study revealed for the first time a strong time dependence of the prognostic information provided by the signature. Indeed, the HR for TDM and overall survival were 13.58 and 8.20 at 5 years, compared with 5.11 and 2.55 at 10 years for...
Recurrence was strongly prognostic in the identification of late metastasis. While proliferation-based gene-expression signatures were initially developed using frozen tumor samples, all of them can now be performed on paraffin-embedded tissue. The Prosigna and MammaPrint assays were approved by the FDA in 2013 and 2007, respectively and by the European Union in 2014 as a prognostic tool to predict distant recurrence in women with stage I/II, node-negative or 1–3 node-positive breast cancer (Prosigna approved only for ER+ patients over the age of 60 years). Although not FDA approved, Oncotype DX is the most widely used prognostic gene-expression signature in the United States. Although these assays are routinely used in clinical practice in some countries, we are still awaiting level-one evidence of their clinical utility. Large clinical trials such as TAILORx (Oncotype DX), MINDACT (MammaPrint), and ASTER 70 (Genomic Grade Index) are ongoing to assess the value of these signatures to guide treatment-related decisions in early breast cancer, either in patients with intermediate risk scores (TAILORx), in patients with discordant clinical and molecular prognosis (MINDACT), or in the elderly population (ASTER 70); the first results should be available in the near future.

Conclusions

The beginning of the third millennium was a turning point in our understanding of breast cancer, with the first description by Perou and colleagues (3) of the molecular subtypes of breast cancer. Concurrently, the first prognostic gene signatures were developed to predict the risk of recurrence in early breast cancer. Since that time, our understanding of the information provided by these tools has been constantly evolving.

Exploration of the first-generation gene signatures revealed the dominant weight of proliferation-associated genes and the strong time dependence of these prognostic assays. Moreover, by analyzing thousands of breast tumor gene-expression profiles, we were able to reveal that the prognostic influence of different biologic processes is dependent on the breast cancer subtype. We showed that the prognostic power of the proliferation-based gene signatures is mostly restricted to ER+/HER2+ breast cancer. We also demonstrated that expression of immune-related genes is associated with outcome in HER2+ and ER–/HER2+ breast cancer, thus providing a rationale for the development of immune-associated biomarkers and immunotherapies in these subtypes. The story of the gene signatures also highlights the ways in which technological progress may radically modify our understanding of the disease. Recent advances now also made high-throughput RNA and DNA sequencing studies possible, as well as cancer proteomic and epigenetic analyses. Likewise, refinement of the sequencing platforms now enables molecular analysis directly in the blood of cancer patients by interrogating CTCs, ctDNA, and circulating microRNA, which allow for real-time monitoring of tumor molecular evolution (34). All of these platforms represent many windows that reveal different aspects of cancer biology. Hopefully, future research will enable translation of this incredible knowledge into meaningful clinical benefit for cancer patients.

Disclosure of Potential Conflicts of Interest

C. Sotiriou is listed as a co-inventor on a patent, which is owned by the Université Libre de Bruxelles, related to the Genomic Grade Index, a prognostic gene-expression signature. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: I. Gingras, C. Desmedt, M. Ignatiadis, C. Sotiriou Writing, review, and/or revision of the manuscript: I. Gingras, C. Desmedt, M. Ignatiadis, C. Sotiriou

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