Molecular profile in breast cancer

Francesco Pantano[1], Loretta D'Onofrio[1], Aleix Prat[2,3]

Abstract
Over recent decades breast cancer research has undergone a real revolution thanks to the emergence of novel technologies based on high throughput gene expression analysis. Gene-expression profiling has made a significant contribution to our understanding of breast cancer biology, and clinical researchers have moved from "semantic" classification of breast cancer subclasses by pathology-based biomarkers (e.g. estrogen receptor, progesterone receptor and HER2 status) into new genomic classifiers. The terminology of intrinsic subtypes was adopted by the 2011 St. Gallen Consensus Conference to describe the paradigm for making treatment decisions in patients with breast cancer. Apart from expanding prognostic knowledge, the application of molecular profiling will allow prediction of treatment efficacy and forecasting of outcomes for individual patients with breast cancer. Currently available tests include Oncotype DX™, NanoString PAM50 test (Prosigna®), MammaPrint® and EndoPredict. Prospective clinical trials adding these tests are currently ongoing.

Keywords: breast cancer, gene expression, molecular subtype, PAM50

Introduction

Breast cancer was historically seen as a single disease with varying histopathological characteristics and response to systemic treatment. Clinico-pathologic features, such as tumor size, histological grade and number of metastatic axillary lymph nodes (prognostic tools), were evaluated together with estrogen (ER) and progesterone receptor (PR) expression, human epidermal receptor (HER) 2/neu-amplification and proliferation index Ki67 (predictive tools) in order to estimate the probability of breast cancer recurrence and predict survival. In most cases, patients with ER/PR-positive early-stage breast cancer are offered endocrine therapy, and those with HER2-positive tumors are offered HER2-targeted therapies. However, determining which patients would benefit from adjuvant chemotherapy is a much more complex decision, especially considering the risk of unnecessary side effects in over-treated patients. Biomarkers to predict the benefit of chemotherapy are limited, meaning that the indication for chemotherapy treatment is based on prognosis alone [1].

It is now well established that breast tumors also have intrinsic molecular patterns that can provide information about the potential of biologic therapies. At least five different biologic subtypes are recognized: luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like [2]. It is also true that the exact number of molecular subclasses of breast cancer is currently unknown. Currently, up to 30% of cases do not fit into any of the recognized four molecular categories and, as genomic studies evolve, new molecular classes are being defined, such clauding-low in basal-like disease [3]. Each intrinsic subclass is well plotted to an immunohistochemistry (IHC)-defined subtype except for normal-like tumors (7.8% of all breast tumors), which share a similar IHC status with the luminal A subtype and are characterized by normal breast tissue profiling. Microarray-based gene expression profiling studies acted as a springboard for the "new" molecular characterization of breast cancer. Landmark studies undertaken by Perou et al. [2] and Sorlie et al. [4] first clarified that ER-positive and ER-negative breast cancers are fundamentally distinct diseases in molecular terms and that the currently used pathological-based biomarkers (e.g. ER, PR, HER2 and Ki67 or grade) are not able to fully reflect the intrinsic subtypes of breast cancer [5].

Several multigene predictor kits that are now available and have been endorsed by the American Society of Clinical Oncology, St. Gallen and National Comprehensive

[1] Department of Medical Oncology, University Campus Bio-Medico, Rome, Italy.
[2] Department of Medical Oncology, Hospital Clínic of Barcelona, Barcelona, Spain.
[3] Translational Genomics and Targeted Therapeutics in Solid Tumors, August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain.
Correspondence to:
Francesco Pantano MD, PhD
University Campus Bio-Medico,
Medical Oncology Department/Traslational Oncology Laboratory,
Via Alvaro del Portillo 200, 00128 Rome, Italy.
Phone: +39 06 225419117 – Fax: +39 06 225411208
E-mail: f.pantano@unicampus.it
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Cancer Network guidelines as information that could assist therapeutic decision-making in ER-positive cancers. This article provides an overview of recent advances in defining molecular classification of breast cancer.

Breast gene expression-based assays

There are many gene-expression prognostic signatures available to help clinicians predict outcomes for individual patients with breast cancer and to help identify which patients could safely be excluded from adjuvant chemotherapy. First-generation assays (i.e. MammaPrint®, Oncotype DX™) identify poor prognosis diseases by the degree of expression of proliferation-related genes on epithelial cancer cells [6] and recognize ER-related genes and proliferation markers as the two most powerful molecular processes associated with outcome. As a result, prognostic information is mostly confined to ER-positive tumors within the first 5 years from diagnosis. In order to better estimate long-term prognosis in ER-positive (or negative) diseases, second-generation prognostic signatures (i.e. Prosigna®, EndoPredict) incorporate expression of genes related to immune response, stromal cells, and cancer-related pathways with a better prognostic value for late recurrences [7]. The two most successful tests commercially are Oncotype DX™ and MammaPrint®, and recently the NanoString PAM50 test, marketed as Prosigna Breast Cancer® [8]. Characteristics of the main commercially available gene-signature assays are summarized in Table 1.

Oncotype DX™

Oncotype DX™ (Genomic Health, Redwood City, CA, USA) is a quantitative reverse transcription-polymerase chain reaction (PCR)-based assay, reported as a numerical score (recurrence score, RS) ranging from 0 to 100. Based on measuring the expression of 16 cancer-related genes and five reference genes, the Oncotype DX™ assay defines three prognostic categories: low-risk (<18), intermediate-risk (18–30), and high-risk (≥31) [9]. This assay was first developed in ER-positive, HER2-negative, node-negative breast cancer patients who had been randomized to the tamoxifen-only arm of the National Surgical Adjuvant Breast and Bowel Project B-20 trial. Subsequently, the test's prognostic value was tested in 3 independent studies, the NSABP-20, NSABP-14 and ATAC trials [10], all of which showed that the Oncotype DX™ RS was able to predict distant recurrence in patients with ER-positive, node positive or negative early breast cancer. The ability of Oncotype DX™ RS to predict response to chemotherapy was evaluated in the NSABP B-20 trial (CMF *vs* CEF regimen) and the Southwest Oncology Group (SWOG)-8814 trial (CAF regimen) [11]. Both retrospective analyses showed that patients with a high RS obtained significant benefit from chemotherapy, while those with low or intermediate RS did not experience an improvement in disease-free survival (DFS) when treated with chemotherapy.

Interestingly, changing of the treatment recommendation after clinicians received Oncotype DX™ assay results (from endocrine to chemotherapy or vice versa) was assessed in at least 20% of patients. The Trial Assigning Individualized Options for Treatment (Rx) (TAILORx) was conducted to provide prospective validation and refine the clinical usefulness of the 21-gene Oncotype DX™ assay in a specified low-risk cohort of women with hormone receptor-positive, HER2-negative, axillary node-negative invasive breast cancer [12]. In September 2015,

Table 1. Summary of assay characteristics.

Assay name	Gene (n)	Clinical material	Platform	Training parameter	Test results	Randomized prospective trials
PAM50 [12] (research based + Prosigna®)	55	FFPE and fresh	qRT-PCR and microarray	Biology (identification of major molecular subtypes)	Intrinsic subtypes + ROR (low, medium, or high)	RxPONDER (1–3 nodes, recruiting; embedded additional analysis)
MammaPrint® [17]	70	FFPE	Microarray	Outcome (5-y metastasis rate in pM0 ER+ women)	Good-bad	MINDACT prognosis validation
Oncotype DX™ [8]	21	FFPE	Reverse transcriptase-PCR	Outcome (recurrence in mainly tamoxifen treated in ER+ pN0 women)	Low, intermediate or high	TAILORx RxPONDER (1–3 positive nodes, recruiting)

ER+: estrogen receptor-positive; FFPE: formalin-fixed paraffin-embedded; qRT: quantitative real time; PCR: polymerase chain reaction; pM0: no metastases; pN0: no regional lymph node involvement; ROR: risk of recurrence.

results were reported from an analysis of the women in the low-RS risk group (treated with hormone therapy alone). Five-year rates for distant relapse-free, invasive disease-free and overall survival were 99.3%, 93.8% and 98.0%, respectively. These results provide prospective evidence that the gene expression test identifies women with a low risk of recurrence who can be spared chemotherapy. Ongoing trials as RxPONDER (NCT01272037) are evaluating whether adjuvant chemotherapy is beneficial in patients with hormone-receptor-positive, HER2-negative breast cancer with positive axillary lymph nodes (1–3 positive nodes) and a recurrence score of 25 or less. The most important limitation for Oncotype DX™ assay is that it has only been validated in hormone receptor-positive breast cancer, and there are no data on the utility of this test for other breast cancer subtypes. Moreover intermediate-RS risk group appears uninformative in about one-third of patients.

NanoString PAM50 test – marketed as Prosigna®

PAM 50 is a 50-gene expression assay (plus 5 control genes) based on microarray and quantitative real time (qRT)-PCR that is able to define intrinsic molecular subtype (luminal A, luminal B, HER2-positive and basal-like), and provide a risk of relapse score for stage I/II (including one to three positive nodes), ER-positive breast cancer in postmenopausal women [13]. The PAM50 test was adapted to be performed using the nCounter Analysis System in order to develop a simplified workflow that could be performed in a local pathology lab in frozen or formalin-fixed paraffin-embedded (FFPE) tissues (Prosigna® Breast Cancer Gene Signature Assay, NanoString Technologies, Seattle, WA, USA). The PAM50/Prosigna® Risk of Recurrence (ROR) score classifies patients as high, intermediate, or low risk and is generated based on an algorithm that incorporates the 50-gene signature, intrinsic subtype, and tumor size [14]. Results from the Austrian Breast and Colorectal Cancer Study Group Trial 8 (ABCSG-8), which enrolled tamoxifen-treated, postmenopausal, ER-positive women, indicated that the Prosigna® ROR was able to predict late distant recurrence [15]. RNA extracted from tumor blocks prospectively collected in the TransATAC trials showed that PAM ROR scores were able to predict late distant recurrence for patients with one positive node as well as those with two or three positive nodes [16]. The same trials compared the Oncotype DX™ RS with the PAM50 ROR score. Both assays were found to provide additional prognostic information beyond clinical-pathological features (nodal status, tumor size, histopathologic grade, age) but the ROR score provided more prognostic information than the RS score for late recurrence (in years 5 through 10) [17]. The intrinsic subtype determination of the Prosigna® assay has also been validated in ER-negative or HER2-positive patients, however, clinical utility in these subgroup is unknown. Looking at predictive value, the RxPONDER trial has been designed to evaluate the clinical utility of Oncotype DX™ for one to three node-positive patients; however, the Prosigna® assay will be conducted on tumor samples as a secondary risk assessment tool and may provide Level I evidence supporting the predictive ability of Prosigna®. Similar to RxPONDER, the OPTIMA study (ISRCTN42400492) compares the management of patients using test-directed assignment to chemotherapy with standard management (chemotherapy) in a non-inferiority design (the main trial will use the Prosigna® assay).

MammaPrint®

In 2007, the MammaPrint® assay became the first multigene profiling assay to obtain FDA approval for breast cancer patients younger than 61 years of age with stage I/II, lymph node-negative or one to three lymph node-positive disease, irrespective of hormone receptor or HER2 amplification status. High *versus* low risk groups are stratified by using a 70-gene signature, first validated in a series of 295 consecutive invasive breast tumors from patients with early stage breast cancer who were all part of the tumor bank at the Netherlands Cancer Institute (NKI) [18]. A large prospective trial (MINDACT) tested the clinical utility of MammaPrint® and accrued 6,693 patients between 2006 and 2011: among women categorized as having a high clinical risk of breast cancer recurrence (defined by common clinical and pathological criteria) but low genomic risk according to MammaPrint® assay, withholding chemotherapy resulted in a 1.5% reduction in 5-year survival without distant metastasis compared with chemotherapy alone. On this basis, approximately 46% of women with breast cancer who are at high clinical risk might not actually require chemotherapy [19]. A longer follow-up is recommended to confirm long-term survival benefit when a "genomic" strategy is preferred by clinicians over a "clinical" strategy.

EndoPredict

EndoPredict is an 11 gene-based assay that was developed to predict distant recurrence in patients with ER-positive, HER2-negative breast cancer receiving adjuvant endocrine therapy [20]. The EndoPredict (EP) risk score ranges from 0 to 15, with higher values indicating a higher risk of recurrence. The EP score has also been combined with

nodal status and tumor size to compute a comprehensive risk score termed EPclin. EndoPredict prognostic ability was retrospectively validated in RNA extracted from tumor blocks prospectively collected for the ABCSG-6 and ABCSG-8 trials and now in the TransATAC trial [21]. In the TransATAC cohort, both EP and EPclin were highly prognostic across the 10 years of follow-up, providing more prognostic information than Oncotype RS™.

Molecular profile in breast cancer: clinical relevance

If considering any form of recurrence, Oncotype DX™, Prosigna®, MammaPrint® and EndoPredict have all demonstrated evidence supporting their prognostic role. In daily clinical practice, genomic based-assays become helpful if used appropriately, or, in other words, if employed in order to better assess prognosis. In fact, they can potentially reduce overtreatment through the selection of individuals not suitable for chemotherapy when standard clinico-pathologic features would have suggested otherwise (i.e. luminal A *versus* luminal B according to Ki67 index by IHC). This scenario is particularly important in women with hormone receptor-positive, HER2-negative, node-negative (or 1–3 positive) tumors, who would gain little or no benefit from adjuvant treatment. The American Society of Clinical Oncology (ASCO) has put out new guidelines on using such biomarkers to make decisions about systemic therapies after surgery in early-stage invasive breast cancer [22]. More data about long-term prognostic value are warranted, especially regarding recurrence that occurs more than five years after surgery (late recurrence); the prognostic ability of the Oncotype DX™ RS is well established in the first five years, but it was not significant in years 5 through 10. Both the Prosigna® and EndoPredict assays appear to possess better prognostic value for late recurrences while also remaining predictive of early relapse. It is also true that there are no clinically useful prognostic signatures for ER-negative or HER2 positive cancers, and drug-specific treatment response predictors also remain elusive.

A strength of gene-expression-based assays is the high level of standardization and the possibility of overcoming inter-observer and inter-laboratory variability of results with subsequent high reproducibility.

Challenges include the necessary budget, need for a central laboratory (except for EndoPredict or Prosigna® assays), tumor sample collection (e.g. contamination with other cell types, tumor heterogeneity, aneuploidy), potential to outperform conventional clinico-pathological parameters, and integration of host and microenvironment information.

Regarding the concordance in risk prediction among the different tests has raised, comparative studies indicate that discordant risk prediction frequently occurs when different prognostic assays are applied to the same case, even if they result in a similar prognostic value [23].

Conclusions

Unquestionably, microarray studies undertaken over the last decade have provided deeper insight into the complex biology of breast cancer. We are not yet ready to leave behind the decision-making process based on traditional clinico-pathologic features (age, tumor size, node status, and hormonal and HER2 status) and the new molecular tools may be used in conjunction with these classical parameters. The clinical utility of multigene profiling assays is currently established for an appropriate subset of patients with ER-positive, HER2-negative, node-negative breast cancer in which the decision to give chemotherapy is difficult to make. Biological knowledge obtained from gene expression profiling studies, however, will prove useful for research into new types of biomarkers. A truly predictive chemotherapy genomic signature for breast cancer would likely be best developed in the neoadjuvant setting correlating signature with pathologic complete response, which is a validated surrogate marker for overall survival. Emerging areas of research involve the development of immune gene signatures that carry modest but significant prognostic value independent of proliferation and ER status, and represent candidate predictive markers for immune-targeted therapies. Looking to breast cancer from a genomic point of view offers a new approach for predicting an individual patient's prognosis by interpreting the expression pattern of a panel of specific tumor-related genes, a so-called genomic signature. The correct estimate of risk of recurrence can avoid unnecessary or ineffective treatments, including cytotoxic chemotherapy, and helps to answer to two fundamental questions: "Should adjuvant treatment be prescribed?" and "Which type of adjuvant treatment should be prescribed?".

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Conflicts of Interest

The Authors declare there are no conflicts of interest in relation to this article.

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