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Review

Gene expression profiling in breast cancer: A clinical perspective

Grazia Arpino [a,*], Daniele Generali [i], Anna Sapino [b], Lucia Del Matro [c], Antonio Frassoldati [d], Michelino de Laurentis [a], Paolo Pronzato [c], Giorgio Mustacchi [e], Marina Cazzaniga [f], Sabino De Placido [a], Pierfranco Conte [d], Mariarosa Cappelletti [h,i], Vanessa Zanoni [h,i], Andrea Antonelli [h,i], Mario Martinotti [i], Fabio Puglisi [h], Alfredo Berruti [g], Alberto Bottini [i], Luigi Dogliotti [g]

[a] Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università di Napoli Federico II, Napoli, Italy
[b] Department of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy
[c] Department of Medical Oncology, National Institute for Cancer Research, Genoa, Italy
[d] Department of Oncology, Hematology and Respiratory Diseases, Modena University Hospital, Modena, Italy
[e] Centro Oncologico, Università di Trieste, Trieste, Italy
[f] Medical Oncology Unit, S. Gerardo Hospital, Monza, Italy
[g] Oncologia Medica, Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, TORINO, Italy
[h] Department of Oncology, University Hospital of Udine, Udine, Italy
[i] Unità Operativa Multidisciplanare di Patologia Mammaria/Breast Unit, Dipartmento di Chirurgia, Azienda Istituti Ospitalieri di Cremona, Italy

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ABSTRACT

Gene expression profiling tests are used in an attempt to determine the right treatment for the right person with early-stage breast cancer that may have spread to nearby lymph nodes but not to distant parts of the body. These new diagnostic approaches are designed to spare people who do not need additional treatment (adjuvant therapy) the side effects of unnecessary treatment, and allow people who may benefit from adjuvant therapy to receive it. In the present review we discuss in detail the major diagnostic tests available such as MammaPrint dx, Oncotype dx, PAM50, Mammostrat, IHC4, MapQuant DX, Theros-Breast Cancer Gene Expression Ratio Assay, and their potential clinical applications.

Introduction

A number of prognostic and predictive factors predict for future recurrence or death from breast cancer. The strongest prognostic factors are patient age, comorbidity, tumor size, tumor grade, number of involved axillary lymph nodes, and possibly biomarker status (e.g., HER2, estrogen, and progesterone receptors). Algorithms have been published estimating rates of recurrence and a validated computer based model (Adjuvant! Online for breast cancer)[1,2] is available to estimate 10-year disease-free survival that incorporates all of the above prognostic factors except for HER2 tumor status. Guidelines from professional societies, such as the St Gallen International Breast Cancer Expert Panel, The National Institute of Health (NIH) Consensus Criteria,[3] the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN), have recommended that the decision to use systemic adjuvant therapy requires considering balancing risk of disease recurrence with local therapy alone, the magnitude of benefit from applying adjuvant therapy, toxicity of the therapy and comorbidity.[4,5]

Gene-expression profiling studies have led to an innovative molecular classification of breast cancer into four distinct subtypes[6]: the basal-like subtype, which is estrogen receptor (ER)-negative and HER2-negative; the HER2 subtype, characterized by increased expression of HER2 and of genes mapping to the HER2 amplicon; and two luminal ER-positive subtypes: luminal A, characterized by high levels of ER and ER-related genes, and luminal B, characterized by lower ER levels and high expression of genes implicated in the proliferation process. These newly defined molecular subgroups have distinct clinical outcomes.[7–9] Luminal A tumors are extremely sensitive to endocrine therapy and have a more favorable natural history than basal-like and HER2-like tumors notwithstanding the greater sensitivity of the latter tumors to chemotherapy.[10]

* Corresponding author.
E-mail address: grazia.arpino@unina.it (G. Arpino).

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The aim of gene-expression profiling technology is to provide a better prediction of clinical outcome than the traditional clinical and pathological parameters. This tool has been developed to further aid clinician in objectively estimating outcome with local treatment only, and also assist in estimating the absolute benefits expected from systemic adjuvant endocrine therapy and chemotherapy. However, the identification of low-risk patients not needing adjuvant chemotherapy, and tailoring therapy in relation to the RNA transcripts produced by cancer cells remains a challenge. In this paper, we review the gene expression signatures currently commercially available and discuss their limits and applicability to clinical practice in terms of personalized treatment.

Available tests, technical issues and feasibility

Tumor gene signatures were initially developed to help clinicians address the two main questions related to the management of breast cancer patients: "Should adjuvant treatment be prescribed?" and "Which type of adjuvant treatment should be prescribed?". Among the computerized tools devised to address these challenges, Adjuvant! Online is probably the most popular (www.adjuvantonline.org). Similarly, many molecular analyses that explore tumor gene signatures have been reported to be prognostic or predictive of the clinical outcome of breast cancer, and easy to incorporate in routine clinical practice.[11,12] However, before entering into routine use, it should be demonstrated that these novel gene predictors really add new independent information, and that they are reliable tools for decision-making at an individual level.[13] Finally, given the costs of these tests, we should evaluate in how many cases the gene predictors could change our practice, and whether they are cost-effective on a large scale.

Methods have been proposed to grade the evidence used in stratifying cancer risk to accommodate newer study designs that are emerging as a consequence of biomarker development. The efficacy of new tests is usually evaluated based on their clinical validity and clinical utility. Clinical validity defines the ability of the test to accurately and reliably identify or predict the intermediate or final outcomes of interest.[14] This is usually reported as clinical sensitivity and specificity. Clinical utility defines the balance of benefits and harms associated with the test, and should include improvement in measureable clinical outcomes and use.

In the present review, we describe the potential clinical uses of the currently available gene signature tests and their clinical validity as reported in the studies available (Table 1).

Methods

Identification of published reports

Studies were identified by a computerized search of the Medline (1966–2012), Cancerlit (1966–2012), and Embase (1990–2012) databases using the following text words: "gene arrays, breast cancer, gene expression profiling, MammaPrint, Oncotype DX, Mammostrat, Immunohistochemistry panel, Recurrence score, Theros, Genomic Grade Index, MapQuant, PAM50, Breast Bio-Classifier". We limited the search to English-language articles on human research that were published between 1966 and February 2012. A computerized search of the proceedings of the annual meetings of the American Society of Clinical Oncology (ASCO) held between 1998 and 2012 was also run to identify relevant studies published in abstract form. Lastly, all review articles and all cross-referenced studies from retrieved articles were screened for further pertinent articles.

Table 1
Gene predictor tests available in the clinic setting.

Test	Reference	Company	Tissue requirements	Technique	Output/Score
MammaPrint® (FDA approved)	van't Veer LJ et al., Nature 2002	Agendia BV, (Amsterdam, Netherlands)	Tissue core sampled on fresh specimens to be preserved in RNA later and immediately sent to the company; as an alternative, frozen archival material.	Microarray-based gene expression profiling	**2 Categories of** tumors with different **risk** to develop metastasis at 10 years - **low-risk** tumors (13%) - **high-risk** tumors (56%)
Oncotype DX™	Paik S et al., N Engl J Med 2004	Genomic Health Inc. (Redwood City, CA, USA)	Either fresh frozen or FFPE archival tissue	qRT-PCR (21 genes)	**Recurrence score** (0–100): predicts the risk of 10-year distant recurrence in ER-positive, lymph node negative patients - **low** (<18) - **intermediate** (18–31) - **high** (≥31)
Theros-Breast Cancer Gene Expression Ratio Assay®	Ma XJ et al., Cancer Cell 2004	Biotheranostics (Biomérieux Alliance Groupe, San Diego, USA)	Either fresh frozen or FFPE archival tissue	qRT-PCR (3 genes)	**HOXB13: IL17R ratio** stratifies ER-positive breast cancer into low or high risk for recurrence and is predictive of benefit from endocrine therapy
PAM50/Breast BioClassifier™	Parker JS et al., J Clin Oncol 2009	University Genomics, Inc./ARUP Laboratories	Either fresh frozen or FFPE archival tissue	qRT-PCR (55 genes)	**Continuous risk of recurrence**
MapQuant Dx™	Sotiriou C et al., J Natl Cancer Inst 2006 and Toussaint J et al., BMC Genom 2009	Ipsogen (Breast Cancer Profiler)	Either fresh frozen or FFPE archival tissue	qRT-PCR (8 genes)	**Genomic Grade Index** Divides histologically defined G2 tumors into: - GGI low-grade - GGI high-grade
Mammostrat®	Ring BZ et al., JCO 2006	Applied Genomics, Inc., (Huntsville, Alabama)	Either fresh frozen or FFPE archival tissue	IHC (5 proteins by 5 monoclonal abs)	**Mammostrat risk score**: high, moderate, or low risk of recurrence after tamoxifen treatment

Abs: antibodies; FFPE: formalin fixed paraffin embedded; GGI: Genomic Grade Index; IHC: immunohistochemistry; RT–PCR, reverse-transcriptase polymerase chain reaction.

Quality evaluation

Studies were divided according to the types of test and the nature of study (molecular/laboratory research or clinical). The relevance and reproducibility of the methods and findings and the number of samples analyzed in each study were the most important parameters in evaluating the quality of the data. Whenever possible, especially when addressing treatment and clinical management issues, we gathered data from large scale prospective randomized trials with clinically important endpoints as disease free survival, (DFS) or overall survival (OS) because these studies have the most rigorous designs and provide the most useful information. For clinical studies, quality was based on the sample size and the rigor of the study design.

MammaPrint dx

Clinical validity

Using gene expression profiling, Van't Veer and colleagues developed a 70-gene classifier, the MammaPrint dx signature, that accurately distinguished breast cancer patients who were likely to remain free of distant metastases (good profile) from breast cancer patients at high risk of developing distant metastases (poor profile) within the 5 years after diagnosis.[15] To develop this 70-gene profile, 78 tumors from women with lymph node-negative breast cancer were studied. Patients were under 55 years of age at diagnosis, had a primary invasive breast carcinoma less than 5 cm in diameter, no previous malignancies and were treated at The Netherlands Cancer Institute (NKI).

MammaPrint was next validated in a series of 295 consecutive (to rule out selection bias) women with breast cancer collected according to a NKI protocol.[15] The samples of patients were from the NKI tumor bank, and included all patients observed at the NKI since 1986 with a diagnosis of early breast cancer. In a subset of 151 patients with lymph node-negative disease (95% of whom received no adjuvant chemotherapy), the proportion of patients who remained free of distant metastases at ten years was 87% in the "low risk" group and 44% in the "high risk" group. The gene profile was a statistically independent predictor of outcome and added to the power of standard clinico-pathologic parameters; hazard ratio (HR) 4.6 (95% CI 2.3–9.2).

The TRANSBIG Consortium conducted the second independent validation study for MammaPrint.[16] The five European hospitals of the Consortium enrolled 302 untreated patients with at least 10 years of follow-up. The proportion of patients who remained free of distant metastases at ten years was 88% in the "low risk" group and 71% in the "high risk" group. MammaPrint was found to provide prognostic information beyond what could be determined from patient age, tumor grade, tumor size, and ER status in a population of node-negative patients, none of whom received any adjuvant endocrine or chemotherapy. The MammaPrint test performed better than Adjuvant! Online in predicting the outcome of patients. Discordance rates between the two tests were 28% and 35% in the "low" and "high" risk groups respectively, which indicates that the tests had totally independent predictiveness. However, the clinical outcome of the discordant cases were most accurately predicted by MammaPrint. In fact, 34% of Adjuvant Online! "high risk" patients could have avoided chemotherapy because they had "low risk" MammaPrint profiles. Moreover, 14% of Adjuvant Online! "low risk" patients had "high risk" MammaPrint profiles and required adjuvant treatment based on outcome data.

These results highlighting the independent prognostic factor in node-negative breast cancer patients were confirmed in several subsequent studies.[17] A study from the Massachussetts General Hospital[18] including 100 older American breast cancer patients showed that MammaPrint has an excellent negative predictive value (NPV) correctly identifying 100% of women at low risk for distant metastases at 5 years after adjuvant treatment. However, in this study the assay had a lower positive predictive value (PPV) (12% at 5 years) than previously observed.[18] Additional work demonstrated that MammaPrint has a strong prognostic value in patients with 1, 2 or 3 positive lymph nodes[19] and in patients over 55 years.[20] In summary, MammaPrint provides a dichotomous (binary) test result: Low Risk versus High Risk of developing distant metastases and sensitivity of the test exceeded 90% in over 97% of patients.

Potential clinical use

MammaPrint is effective in distinguishing patients with a "good" prognosis from patients who develop early metastases. The hazard ratios for MammaPrint are exceptionally high in the first 5 years following curative treatment; indeed, they ranged from 4.5 to 4.7 for time-to-distant metastasis adjusted for clinical risk.[16] It is noteworthy that chemotherapy exerts its maximal beneficial effect during the 5 years post-treatment.[21] Risk of recurrence was clearly lower in patients who received adjuvant treatment than in untreated patients in this same 5-year period, whereas after this interval the difference in risk of recurrence stabilizes. For treatment with anthracycline-based chemotherapy, this benefit may even be restricted to the first 2 years following treatment.[22] MammaPrint was developed to distinguish patients who are likely to develop metastasis in the time frame that overlaps chemotherapy benefit. Bender et al.[23] and Knauer et al. 2009[24] conducted a meta-analysis of 7 studies for a total of 1696 patients with a median follow-up of 7.1 years. Of these, 315 received hormonal therapy alone and 226 received hormonal therapy plus chemotherapy. Adjuvant chemotherapy was CMF or an anthracycline with or without taxane-based chemotherapy. MammaPrint assigned 252 (47%) patients to "low risk" and 289 (53%) patients to "high risk" of recurrence.

In the MammaPrint "high risk" group, there was a significant (HR 0.35, $P < 0.01$) benefit for the combined treatment of 12%. These results remained robust in a multivariate analysis (HR 0.38, P 0.04). Conversely, there was no significant benefit for hormonal therapy plus chemotherapy versus hormonal therapy alone in the "low risk" patient group.

Several studies have focused attention on the reproducibility and reliability of microarray measurements showing that, microarray technology can be used as a reliable diagnostic tool given the high intralaboratory and interlaboratory reproducibility when using strictly controlled standard operating procedures.[25,26]

The MammaPrint test is intended for use in women 61 years of age or younger with primary invasive (stage I or II) breast cancer who are lymph node-negative and have a <5 cm, ER-positive or ER-negative tumor. MammaPrint was cleared for marketing by the U.S. Food and Drug Administration (FDA) in 2007 for use as a prognostic test to be used with other clinicopathologic factors. The test must be done on frozen fresh tumor tissue/fresh tissue in RNAlater or formalin-fixed and paraffin-embedded (FPE) tissue specimens and its results are reported as low risk ("13% chance of developing distant metastases at 10 years without adjuvant treatment") or high risk ("56% chance of developing distant metastases at 10 years without adjuvant treatment").

Genomic health recurrence score (GHI-RS) Oncotype DX (Oncotype DX Breast Cancer Assay)

Clinical validity

The Oncotype DX Breast Cancer Assay is a commercially available reverse transcriptase-polymerase chain reaction (RT-PCR)-

based signature. It evaluates the mRNA expression levels of only 21 genes (16 cancer-related genes and 5 reference genes).[8] These 16 genes comprise components of the ER pathway (ER, progesterone receptor, BCL2 and SCUBE2), proliferation (Ki67, STK15, Survivin, CCNB1 and MYBL2), HER2 amplicon (HER2 and GRB7), invasion (MMP11 and CTSL2) and GSTM1, CD68 and BAG1. The expression of these 21 genes is reported as a single Recurrence Score (RS), which is a continuous variable ranging between 0 and 100. The test is routinely performed on formalin-fixed and paraffin-embedded (FPE) tissue specimens.

Throughout the last decade many laboratories have shown that mRNA levels in FPE samples can be safely quantified by RT-PCR techniques despite the extensive RNA fragmentation that occurs in tissues so preserved. Indeed, Cronin et al. by analyzing data by RT-PCR in 62 specimens dating from 1985 to 2001, showed that the results were substantially concordant when ER, progesterone receptor (PR), and HER2 receptor status determined by RT-PCR was compared with immunohistochemistry (IHC) assays for these receptors. Furthermore, their results highlighted the advantages of RT-PCR over IHC with respect to quantitation and dynamic range, further supporting the development of RT-PCR analysis of FPE tissue RNA as a platform for multianalyte clinical diagnostic tests.[27] Similar results were obtained in another study by Cobleigh et al.[28] The authors, by analyzing RNA extracted from paraffin blocks of 78 patients with more than 10 metastatic axillary nodes, and quantifying expression of 203 candidate genes by RT-PCR showed that tumor gene expression profiles of archival tissues, some more than 20 years old, provide significant information about risk of distant recurrence even among patients with 10 or more nodes.

The analytical performance of the Oncotype DX were also extensively analyzed by Cronin et al.[29] Their assays used a pooled RNA sample from FPE tissues to evaluate the analytical performance of a 21-gene panel with respect to amplification efficiency, precision, linearity, and dynamic range, as well as limits of detection and quantification. In this analysis, the analytical and operational performance specifications defined for the Oncotype DX assay allowed the reporting of quantitative RS values for individual patients.

Oncotype DX has been validated in several different independent populations using different study designs. The first study population was a subset of patients from a randomized clinical trial, National Surgical Adjuvant Breast and Bowel (NSABP) B-14, that originally included almost 3000 patients randomized to assess tamoxifen benefit in lymph node-negative, ER-positive breast cancer patients.[8] Thus, all study patients received 5 years of tamoxifen therapy. It demonstrated that patients classified as having a low RS (51% of patients) have a significantly different 10-year rate of distant recurrence (6.8%; 95% CI 4.0–9.6) than patients (27%) classified as having a high RS (30.5%; 95% CI 23.6–37.4). However, the low RS group of patients had overlapping confidence intervals with patients (22%) having an intermediate RS (14.3%; 95% CI 8.3–20.3). In the second validation study conducted on 149 patients at the MD Anderson Cancer Center,[30] RS failed to correlate with the 10-year rate of distant recurrence because the confidence intervals of all three groups (high, low and intermediate risk) overlapped. The distant recurrence rate was 18% (95% CI 7–30) in the low risk patients, 38% (95% CI 15–61) in the intermediate risk patients, and 28% (95% CI 13–32) in the high-risk patients. These patients were all untreated. The third validation study was a case control study conducted by Habel et al.[31] in which 220 patients who died from breast cancer were matched with three controls per case (i.e., for a total of 570 breast cancer patients) alive at the time their matched index patient had died. The statistically approximated 10-year recurrence rate was 2.8% (95% CI 1.7–3.9) for patients classified as "low risk" and was statistically different from the "intermediate risk" patients who had a 10-year recurrence rate of 10.7% (95% CI 6.3–14.9). However, patients classified as "high risk" did not differ significantly from "intermediate risk" patients in 10-year recurrence rate (15.5%; 95% CI 7.6–22.8).

Dowsett et al. analyzed the risk of developing a distant recurrence using Oncotype DX in 1308 postmenopausal primary breast cancer patients treated with anastrozole or tamoxifen (Arimidex, Tamoxifen Alone or in Combination, the TransATAC study).[32] In this study, Oncotype DX RS was an independent predictor of the risk of distant recurrence in node negative and node positive HR+ patients treated with anastrozole or tamoxifen, thought it failed to be predictive of a differential benefit between the two different types of endocrine therapy.

A recent study[33] investigated the risk of recurrence and chemotherapy benefit for patients with node-negative, ER-positive breast cancer when calculated with the 21-gene breast cancer assay RS alone or with the RS integrated with pathologic and clinical factors such as tumor size, grade, and patient age (RS-pathology-clinical: RSPC). Patients from the NSABP B-14[34] and the translational research cohort of the TransATAC[32] studies were included in this study if they received hormonal monotherapy, had ER-positive tumors, and RS and traditional clinicopathologic factors assessed (647 and 1,088, patients respectively). The individual patient risk assessments from separate Cox models were combined using meta-analysis to form an RSPC assessment of distant recurrence risk. Risk assessments by the RS and RSPC were compared in node-negative patients. The NSABP B-20 study evaluated the effectiveness of RSPC and RS to predict chemotherapy benefit. The results showed that RSPC had a significantly better prognostic value for distant recurrence than did the RS (P 0.001), and resulted in a better separation of risk. In fact, RSPC classified fewer patients as intermediate risk (17.8% versus 26.7%, P 0.001) and more patients as lower risk (63.8% versus 54.2%, P 0.001) than did RS among 1444 node-negative ER-positive patients. The authors concluded that RSPC refines the assessment of distant recurrence risk and reduces the number of patients classified as intermediate risk. The addition of clinicopathologic measures did not seem to enhance the value of the RS in predicting chemotherapy benefit.

All the above studies have validated that a high RS by the 21-gene RT-PCR assay is predictive of worse prognosis but better response to chemotherapy. Paik et al.[35] further investigated on the possible relationship between the RS and degree of chemotherapy benefit. The RS was measured in tumors from the tamoxifen-treated and tamoxifen plus chemotherapy-treated patients in the NSABP B20 trial. A total of 651 patients were assessable (227 randomly assigned to tamoxifen and 424 randomly assigned to tamoxifen plus chemotherapy). The test for interaction between chemotherapy treatment and RS was statistically significant (P 0.038). Patients with high-RS tumors (i.e., high risk of recurrence) had a large benefit from chemotherapy (relative risk, RR 0.26; 95% CI, 0.13–0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; standard error, SE, 8.0%). Patients with low-RS tumors derived minimal, if any, benefit from chemotherapy treatment (RR, 1.31; 95% CI, 0.46–3.78; absolute decrease in distant recurrence rate at 10 years: mean, 1.1%; SE, 2.2%). Patients with intermediate-RS tumors did not appear to have a large benefit.[35]

In a different analysis, Chang et al.[36] investigated whether tumor expression of the 21-gene RT-PCR assay and other candidate genes can predict response in 97 patients treated with neoadjuvant docetaxel. The authors found a significant relationship ($P < 0.05$) between gene expression and CR for 14 genes, including CYBA. CR was associated with lower expression of the ER gene group and higher expression of the proliferation gene group from the 21-gene assay. Of note, CR was more likely with a high RS ($P = 0.008$).

Potential clinical use

The Oncotype DX Breast Cancer Assay, together with other conventional risk assessment approaches (e.g., tumor staging/grading, analysis of other markers), is intended to predict the likelihood of distant breast cancer recurrence in women of any age with newly diagnosed stage I or II breast cancer, lymph node-negative and ER-positive, who will be treated with tamoxifen. Oncotype DX claims to provide information beyond conventional risk assessment tools, including how likely the woman is to benefit from chemotherapy (CMF) in addition to tamoxifen therapy. The low- (<18), intermediate- (18–30), and high-risk (≥31) categories are reported to correspond to 10-year distant recurrence rates after 5 years of tamoxifen therapy of <12%, from 12% to 21%, and from 21% to 33%, respectively.[37] This test has been recently included in the ASCO and NCCN guidelines for breast cancer treatment, as a predictor of recurrence for ER-positive, lymph node-negative breast cancer patients.[37]

Theros-Breast Cancer Gene Expression Ratio Assay

Clinical validity

In addition to the Oncotype DX Breast Cancer Assay, another quantitative RT-PCR (qRT-PCR) based gene signature expressed in FPE tissue, the H/I and molecular grade index, also known as Theros, produced by Biotheranostics, is currently on the market. Theros is based on the expression of three highly predictive genes: the homeobox gene HOXB13, interleukin 17B receptor (IL17BR) and EST AI240933, identified in a microarray study conducted by Ma and co-workers.[38] It was specifically developed for ER-positive breast cancer patients treated with tamoxifen. In the initial development study of 60 patients, the expression ratio between HOXB13 and IL17BR (H:I ratio) strongly correlated with recurrence, and the test outperformed other clinical pathological prognostic parameters in tamoxifen-treated patients.[38] Subsequently, using qRT-PCR from RNA extracted from FPE tissue, Ma et al.[38] validated the test in a cohort of 20 patients. In another validation study, the two-gene ratio correctly stratified 852 tamoxifen-treated and untreated breast cancer patients into high and low risk.[39] Jerevall et al.[40] in a different study including tumors from 264 randomized postmenopausal patients and 93 systemically untreated premenopausal patients also showed that a high HOXB13:IL17BR ratio was associated with aggressive tumor characteristics, as were low levels of IL17BR alone. The ratio and HOXB13 alone predicted recurrence-free survival after endocrine treatment, with a benefit of prolonged treatment in ER-positive patients correlated to a low ratio (recurrence rate ratio RR = 0.39; $P = 0.030$), or low expression of HOXB13 (RR = 0.37; $P = 0.015$). Jansen et al. measured the HOXB13 and IL17BR expression levels in 1252 ER-positive primary breast tumor specimens to determine the relationship of a HOXB13-to-IL17BR ratio with tumor aggressiveness and/or with response to tamoxifen therapy.[41] In this study, the HOXB13-to-IL17BR ratio was significantly associated with DFS and progression free survival (PFS). Corrected for traditional predictive factors, the dichotomized HOXB13-to-IL17BR ratio was the strongest predictor in multivariate analysis for a poor response to tamoxifen therapy (odds ratio, OR = 0.16; 95% CI, 0.06–0.45; $P < 0.001$) and a shorter PFS (hazard ratio, HR = 2.97; 95% CI, 1.82–4.86; $P < 0.001$). Reid et al.[42] also attempted to validate this model on an independent cohort of 58 patients with resectable ER-positive breast cancer. However, in discrepancy with the above studies, their analyses did not find any statistically significant association between the gene expression of HOXB13, IL17BR or their ratio and outcome after tamoxifen treatment.

Potential clinical use

Theros was originally designed to go beyond the current clinical standard (e.g., ER and PR status) to predict tumor recurrence risk for women on tamoxifen monotherapy, for whom alternative therapies (e.g., aromatase inhibitors, chemotherapy) might be considered.[37] The H:I ratio is a "continuous" marker of recurrence in untreated ER-positive/node-negative patients. The results are reported as a normalized H:I expression ratio together with a categorization of low (roughly 10–27%) or high (roughly 28 to >60%) breast cancer recurrence risk at 5 years.

Genomic Grade Index (MapQuant DX)

Clinical validity

The Genomic Grade Index (GGI) is based on 97 genes that are associated with tumor differentiation and tumor grade ascertained by comparing the expression profiles in histologic grade 3 and histologic grade 1 tumors in a training set of 64 ER-positive tumor samples. The profile has been validated on previously reported cohorts[43] also on publicly available datasets[9] and found to be more closely associated with relapse-free survival than was histological grade.[9] In addition, the GGI appears to reclassify patients with histologic grade 2 tumors into two groups with high versus low risk of recurrence (HR 3.61, 95% CI 2.25–5.78; $P < 0.001$, log-rank test). Another study validated the GGI in 650 ER-positive patients who were untreated or were only treated with tamoxifen. Most of these patients were derived from previously published and publicly available datasets.[44] Furthermore, Liedtke et al.[45] reported that a high GGI is associated with increased sensitivity to neoadjuvant paclitaxel plus fluorouracil, adriamycin, and cyclophosphamide chemotherapy in both ER-negative and ER-positive patients, although it remains a predictor of worse survival in ER-positive patients only. Taken together, these results highlight the importance of tumor-differentiation and tumor-proliferation genes especially in the ER-positive subgroup of patients.

Potential clinical use

The GGI test was designed to characterize high-grade versus low-grade tumors. It can resolve "grade 2" tumors into either "grade 1" or "grade 3" tumors in 80% of cases, and it is the first microarray-based and clinically-validated, molecular diagnostic test to measure tumor grade as an indicator of tumor proliferation, risk of metastasis and response to chemotherapy. The recently developed signature, MapQuant Dx by Ipsogen, is an eight-gene qRT-PCR test[9,46] developed by Sotiriou and colleagues that can be performed on FFEP tissue.[9] in The GGI signature is based on four target genes and 4 are reference genes.[9] As this test reflects genomic grade, it is applicable to all type of carcinomas, although it seems to have limited discriminatory power in ER-negative disease.[11,47]

PAM50/Breast BioClassifier

Clinical validity and potential clinical use

The Breast BioClassifier[48] is a 50-gene qRT-PCR assay that classifies ER-positive and ER-negative breast cancers into subtypes that can predict patient outcome (high, medium and low risk groups). It gives a continuous risk score that can help physicians to make treatment decisions based on estimates of death risk. This tool has some advantages, namely, the use of RT-PCR, the feasibility of paraffin-embedded material, the feasibility to be performed in local

Pathology Units, its applicability to all the subtypes of breast cancer, and not only to ER-positive tumors. PAM50 divides patients in low and intermediate risk groups. Consequently, chemotherapy can be avoided in case of a very good prognosis. The prognostic and predictive significance of intrinsic subtypes identified by both the PAM50 gene set has been investigated by Chia et al.[49] in a recent study. The authors used material from a prospective randomized trial of tamoxifen versus placebo in premenopausal women with primary breast cancer (NCIC CTG MA.12) to evaluate the prognostic and predictive significance of intrinsic subtypes identified by both the PAM50 gene set and by immunohistochemistry. Total RNA from 398 of 672 (59%) patients was available for intrinsic subtyping with the PAM50 test. A tissue microarray was also constructed from 492 of 672 (73%) of the study population to assess a panel of six IHC antibodies to define the same intrinsic subtypes. In this study, classification into intrinsic subtypes by the PAM50 assay was prognostic for both DFS ($P = 0.0003$) and OS (OS; $P = 0.0002$), whereas classification by the IHC panel was not. Luminal subtype by PAM50 was predictive of tamoxifen benefit [DFS: HR, 0.52; 95% CI, 0.32–0.86 versus HR, 0.80; 95% CI, 0.50–1.29 for nonluminal subtypes], although the interaction test was not significant ($P = 0.24$), whereas neither subtyping by central immunohistochemistry nor by local ER or PR status were predictive. Harvel et al. in a population of previously untreated post-menopausal patients with ER-positive breast cancers treated for 4 months in a neoadjuvant setting with the aromatase inhibitor exemestane alone, or in combination with the antiestrogen tamoxifen showed that the PAM50 genes signature predicted response or intrinsic resistance to neoadjuvant endocrine therapy of ER-positive tumors.[50]

In a different study by Kelly et al.[51] risk assignment by PAM50 Breast Cancer Intrinsic Classifier™ and Oncotype DX RS were compared in 151 ER-positive stage I–II breast cancer patients. The authors found a good agreement between the two assays for high (i.e., luminal B or RS > 31) and low (i.e., luminal B or RS < 18) prognostic risk assignment but PAM50 assigns more patients to the low risk category. About half of the intermediate RS group was reclassified as luminal A by PAM50.

Mammostrat®

To address the need for specialized laboratories to ensure the quality assurance required for gene expression-based assays, Ring et al. designed a multiple marker test using genes based on a readily available technology, namely IHC.[52] They investigated the possibility of developing an IHC test using data from many gene expression studies, and tested 700 gene targets chosen on the basis of gene expression patterns in three patient cohorts of 466, 299 and 344 patients, respectively.[52] Twenty antibodies were found to be significantly associated with patient outcome in the 195/466 ER-positive, node-negative patients from the first training cohort. Several IHC panels were found to have prognostic power and they were subsequently validated in the two independent cohorts of patients. This initial study resulted in a set of 5 antibodies that could be combined and used to predict outcome in ER-positive breast cancer patients. Their first study was underpowered in the node-negative subsets of patients and prompted a further validation study of the five-antibody IHC test using patient samples from the NSABP B-14 and B-20 trials.[53] From the B-14 study (initiated to determine the clinical benefit of adjuvant tamoxifen), subsets of 287 placebo and 550 tamoxifen-treated patients were evaluated, from a total of 1414 and 2615 patients respectively. From the B-20 trial (initiated to determine the clinical benefit of adjuvant chemotherapy added to tamoxifen), subsets of 161 tamoxifen-treated patients and 296 tamoxifen plus chemotherapy treated patients were evaluated from a total of 771 and 1535, respectively. The test classifies patients into low, moderate and high-risk patients, and revealed considerable differences in outcome predictions among age groups. Younger patients classified as low risk still had a 20% risk of disease progression versus only 6% for patients 60 years and older. The high-risk patients treated with chemotherapy had an absolute decrease of 21% in recurrence rate. The age stratification needs to be verified in additional studies. Furthermore, as the test was developed in a predominantly post-menopausal cohort, this IHC test may be population-specific.

The IHC signature is currently available and it is marketed under the name of Mammostrat®. It is based on the expression of 5 genes (p53, NDRG1, CEACAM5, SLC7A5, and HTF9C), which significantly improve prediction of outcome in ER-positive breast cancer patients.[52]

New approach: IHC4

Other studies have investigated the prognostic value of a combined IHC signature. Cuzick et al.[54] recently compared the prognostic value of the combined IHC score (ER, PR, Ki-67 and human epidermal growth factor receptor 2 [HER2]) with that of the mRNA-based, 21-gene Genomic Health Recurrence Score (GHI-RS). Their aim was to determine whether it provided additional prognostic information regarding distant recurrence beyond that obtained from classical clinicopathologic factors (age, nodal status, tumor size, grade, endocrine treatment) in women with early breast cancer. A primary cohort of 1125 ER-positive patients from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who did not receive adjuvant chemotherapy was evaluated with the GHI-RS. Distant recurrence was the primary endpoint, and proportional hazards models were used with sample splitting to control for overfitting. A prognostic model that used classical variables and the four IHC markers (IHC4 score) was created and assessed in a separate cohort of 786 patients. The study showed that the IHC4 score provided independent prognostic information in the presence of classical variables. In sample-splitting analyses, the information provided by the IHC4 score was found to be similar to that provided by the GHI-RS, and little additional prognostic value was seen when the two scores were combined. The prognostic value of the IHC4 score was also validated in a second separate cohort of patients, and the results indicate that the amount of prognostic information provided by the four widely performed IHC assays is similar to that given by the GHI-RS.

Additional gene assays

In the following paragraphs, assays still awaiting FDA approval, on earlier developmental stages or not commercially available are described and reviewed.

The Rotterdam Signature 76-gene panel analyzes fresh frozen tumor samples and classifies early-stage breast cancer patients at low or high risk of developing metastatic disease according to the tumor gene expression signature.[55] The test can be used in node negative breast cancer patients, regardless of age, tumor size and grade, or ER status. The five-year DFS rate was 90%–98% for low-risk and 74%–76% for high-risk patients. The 10-year DFS rate was 94% for low-risk and 65%–72% for high-risk patients. The reported sensitivity of the test ranged from 80% to 93%, the specificity ranged from 40 to 48% and the positive and negative predictive values were 38% and 94% respectively.[56,57] The test is not yet commercially available and the evidence in the published peer-reviewed scientific literature does not support its accuracy and the clinical utility of the Rotterdam Signature test.

Celera Metastasis Score is an RT-PCR based assay testing the expression of 14 genes on FPE tissues in ER-positive, lymph node-negative tumors. Preliminary studies indicate that this test predicts a 3.5-fold difference in risk for disease recurrence between the women at the highest risk and the women at the lowest risk.[28]

Invasiveness Gene Signature™ (IGS, Oncomed Pharmaceuticals, Redwood City, CA) measures the expression of 186 genes to predict prognosis in early breast cancer patients regardless of nodal and hormone receptor status.[58] Liu et al.[58] showed a significant association between the IGS and both overall and metastasis-free survival ($P < 0.001$, for both) in 295 patients with breast cancer, which was independent of the well established clinical and pathological variables. Validation and refinement of the IGS are currently ongoing to establish and exploit its full clinical value.

NuvoSelect is a 76-gene prognostic signature for lymph node-negative breast cancer patients. In the Foekens et al.[56] study, the 76-gene signature was confirmed as a strong prognostic factor in the subgroups of ER-positive patients, pre- and postmenopausal patients, and in patients with tumor sizes 20 mm or smaller. In a different analysis including 300 lymph node-negative, ER-positive breast cancer patients, the 76-gene signature was able to identify the high-risk patients who benefit most from adjuvant tamoxifen therapy.[59]

HER2-Derived Prognostic Predictor (HDPP) is a 158-gene signature based on hierarchical clustering of gene expression data derived from 58 patients with HER2-overexpressing breast cancer.[60] The predictor includes genes associated with immune response, tumor invasion, and metastasis. HDPP has shown to be able to define patient groups with better and worse outcome in HER2-positive breast cancer across multiple independent breast cancer datasets and to identify a sizable HER2-positive group with long disease-free survival and low mortality. Significant correlation to prognosis is also observed in: basal-like, ER-negative, lymph node-positive, and high-grade tumors, irrespective of HER2 status. Among patients with HER2-positive tumors included the Netherlands Cancer Institute data set, the HDPP provided stronger prognostic information than the MammaPrint and the Oncotype DX systems. Importantly however, the HDPP has no prognostic value in luminal A, luminal B, or normal-like subtypes.[60]

17-gene HER2-TIC-enriched signature (HTICS) is a gene signature generated on the basis of differentially expressed genes in tumor-initiating cells versus non-tumor-initiating cells fractions and trained on one HER2-positive breast cancer cohort. HTICS includes up-regulated genes of the S/G2/M transition and down-regulated genes of the immune response. It has shown to be predictive of clinical outcome on multiple independent HER2-positive cohorts of patients. Its prognostic power, independently of other predictors, stratified lymph node-positive HER2-positive breast cancer into low and high-risk subgroups. Among HER2-positive/ER-negative patients, the 10-years OS was 83.6% for HTICS-negative and 24.0% for HTICS-positive tumors (HR = 5.57; $P = 0.002$). Retrospective analyses revealed that patients with HTICS-positive, HER2-positive and ER-negative tumors were resistant to chemotherapy alone but very sensitive to chemotherapy plus trastuzumab. HTICS is, therefore, a powerful prognostic signature for HER2-positive/ER-negative breast cancer that can be used to identify high-risk patients that would benefit most from anti-HER2 therapy.[61]

Breast Cancer Gene Expression Prognosis Profile (BreastOncPx™**)**: BreastOncPx is a 14-gene signature proposed for use in lymph node negative, ER-positive patients to estimate the likelihood of tumor recurrence (Laboratory Corporation of America, 2010). A "metastasis score" (MS) representing fourteen differentially expressed genes was developed and evaluated for its association with distant-metastasis-free survival (DMFS). Tutt et al.[62] reported that, in a set of 279 untreated subjects, the HR of the high risk compared to low risk groups were 4.02 (95% CI 1.91–8.44) for the endpoint of DMFS and 1.97 (95% CI 1.28–3.04) for overall survival after adjustment for age, tumor size and grade. The low and high MS risk groups had 10-year estimates (95% CI) of 96% (90–99%) and 72% (64–78%) respectively, for DMFS and 91% (84–95%) and 68% (61–75%), respectively for overall survival. The authors suggested that as the signature has a predominance of proliferation genes which have prognostic significance above that of Ki-67 status, it may aid in prioritizing future mechanistic studies and therapeutic interventions.

Gene signature relevance in daily clinical practice

Each new test, as well as each new drug, should be applied in the clinical setting if it provides additional benefit over existing tests or if there is a cost/benefit advantage. Emerging evidence suggests that genomic based-assays may be helpful in the clinical setting if used appropriately. In fact, they can reduce overtreatment and can guide "treatment selection" (i.e., selecting individuals for chemotherapy when standard clinicopathologic features would have suggested otherwise, selecting individuals for chemotherapy when clinicopathologic features suggested therapeutic equipoise). Many centers in Western Europe use gene profiling in their clinical routine, through their health insurance reimbursement, to decide how to treat their patients. The results of the daily clinical routine use of different types of gene profiling assays in various centers were reported in the 2011 St Gallen Breast Cancer Conference.[63] The merged data of 92 patients from Belgium, 36 patients from Italy and 66 patients from the Netherlands showed discordance in clinical risk classification between institutes in 31% of patients. MammaPrint low risk profiles were found in 44%, and MammaPrint high-risk profiles in 56% of patients. Furthermore, this study demonstrates high variability in adjuvant strategies between the different European institutes when treatment choice was based on traditional patient- and tumor-related parameters. The use of MammaPrint would potentially have modified adjuvant treatment in about 34% of patients evaluated in the study.[64] A recent review by Hornberger et al.[65] systematically graded the Level-of-Evidence (LOE), defined according to modified Simon et al.[66] and Hayes et al.[67] criteria, in several studies on gene arrays profiling in early breast cancer patients in order to provide newer framework to base clinical recommendations. Applying their revised evidence-grading criteria to literature on gene arrays studies published before 2011, the authors found that the 21-gene recurrence score satisfies the criteria for Level I evidence determination for predicting distant recurrence risk, OS, and response to adjuvant chemotherapy, as well as Level II evidence for predicting risk of local recurrence. The 70-gene signature, 5-antibody IHC panel, and Adjuvant! Online satisfy Level II evidence for predicting risk of distant recurrence and OS. Adjuvant! Online also satisfied Level II evidence for predicting chemotherapy response. Furthermore, according to the authors, there is Level II evidence for superiority of the 21-gene gene recurrence score over Adjuvant! Online to predict both distant recurrence and response to adjuvant chemotherapy[68] and Level III evidence for the ability of the 70-gene signature to predict recurrence risk superior to that of Adjuvant! Online.[16]

In the next section of this review, we will discuss which patients would benefit from gene predictor testing, and evaluate whether the result would influence the treatment decision, the outcome of the patient or use of resources.

Question 1: Should we use gene-predictors to define the need of adjuvant treatment?

Adjuvant treatment is usually recommended when the risk of tumor-related death at 10 years exceeds 5–10%. This figure can be

influenced by the following clinical and histopathological features: age, tumor size, lymph node status, histological type, tumor grade, HER2 status, hormone receptor status, and proliferation. Among gene predictors, as mentioned above, MammaPrint® has been used to obtain information related to prognosis in untreated node-negative patients.[7,15] In a comparison with the widely used computerized tool Adjuvant!, there was a 29% discordance in low and high risk groups.[19] Sixty-eight percent of patients classified as high risk by Adjuvant! were considered to be at a low risk with MammaPrint®, and could have been spared chemotherapy. On the other hand, 32% of patients deemed to be at low risk with Adjuvant! and who did not receive adjuvant therapy, were considered at high risk based on MammaPrint® results. The molecular predictor was more accurate than Adjuvant!; indeed, there was a 10-year survival rate of 89% in patients re-classified as being at low risk, and 69% in those re-classified in the high risk group. Similar findings have been reported in patients with positive nodes.[19]

However, whether or not these signatures have the potential to outperform conventional clinico-pathological parameters, important practical issues need to be addressed before gene expression profiling can be translated into routine clinical use, even as an ancillary test.[69] The first issue to be considered is sample collection. The MammaPrint test requires the collection, shipment, and analysis of fresh frozen or FPE tissue to ensure optimal test performance. Regarding fresh frozen tissue collection, histopathology laboratories routinely deal with formalin-fixed tissues and collection of fresh frozen tissue may be challenging for small peripheral centers as it requires a detailed logistics organization involving clinical oncologists, surgeons, pathologists and technicians.[37]

Recently researchers at the 8th European Breast Cancer Conference (EBCC-8) held in Vienna 2012 presented the follow-up data from 427 patients with early breast cancer who had taken part in a study called RASTER (MicroarRAy prognoSTics in breast cancER). By looking for a particular selection of 70 genes in a tumor, the MammaPrint® test can predict which patients are at low and which at high risk of distant metastasis, selecting which patients could be spared the side effects of chemotherapy without adversely affecting their chances of disease-free survival. In the group classified as low risk by the MammaPrint® test only 15% of the 219 patients received adjuvant chemotherapy as opposed to 81% (169/208) in the group classified as high risk by the MammaPrint® test. The first group had a five-year DDFS rate of 96% compared with 90% in the high risk group. Despite of these interesting results that could have a concrete feedback in oncologists' clinical practice, whether MammaPrint is better than the classical parameters in case of uncertain risk level need more robust data: the MINDACT (Microarray in Node-negative Disease may Avoid ChemoTherapy) will address this issue.[37]

Question 2: Should we use gene predictors to guide treatment choice, particularly to understand if an ER-positive tumor needs chemotherapy in addition to hormone therapy?

The guidelines available are not very informative in terms of whether or not an ER-positive tumor needs chemotherapy as well as hormone therapy. The NCCN gives indication for chemotherapy (other than hormones) in case of node positivity, and suggests that the Oncotype DX be performed in case of negative nodes with T >1 cm. The St. Gallen recommendations favor chemotherapy if a tumor is greater than 5 cm or if 4+ metastatic nodes are present. What should be done if a tumor is between 2 and 5 cm, or if only 1–3 nodes are positive? In such cases "validated multigene tests, if readily available, could assist in deciding whether to add chemotherapy, after consideration of conventional markers".[5]

Oncotype DX was developed in lymph node-negative, ER-positive untreated breast cancer patients (NSABP B14),[8] and thereafter in the same type of patients treated with tamoxifen (NSABP B14), and again applied to evaluate the effect of chemotherapy (CMF) over tamoxifen in node-negative patients (NSABP B20)[35] and of chemotherapy (CAF)[70] over tamoxifen in node-positive patients. All these retrospective analyses showed that only high risk patients seem to benefit from the addition of chemotherapy, and that the addition of chemotherapy provides no evident increase in disease-free survival in patients with low and intermediate RS. A low RS is predictive of tamoxifen benefit in hormone-positive node-negative cases, whereas a high RS is predictive of chemotherapy benefit over hormonal therapy in hormone receptor-positive patients, regardless of lymph node status. It has been estimated that this information could change treatment recommendation in about 30% of patients. It is important to note that, in contrast to MammaPrint®, Oncotype DX® still has a gray zone, namely, the management of patients with intermediate RS.[37]

Are these results sufficient to endorse the use of Oncotype DX® when we have to decide whether to administer chemotherapy instead of hormonal therapy? This type of test could have some possible advantages in the short term, but, even if all the gene signatures have been shown to be potentially useful mostly in ER-positive, low-proliferating tumors, nodal status and tumor size maintain their independent prognostic value[12] and could be sufficient to for treatment decision making. Moreover, prospective validation of the influence of the use of multigene predictors on the outcome of the patient is required. The ongoing TAILORx trial (Trial Assigning IndividuaLized Options for Treatment) (http://www.cancer.gov/clinicaltrials/digestpage/tailorx) aims to determine the benefit of chemotherapy in patients with an intermediate RS (between 11 and 25, almost 45% of all subjects), while patients with a high RS (>25) will receive chemotherapy plus hormonal therapy, and patients with a low RS (<11) will receive hormonal therapy alone. However, one important limitation of the TAILORx study design is to not provide any data about the cost-effectiveness of Oncotype DX over the conventional prognostic factors in clinical management of breast cancer patients. MINDACT trial design, on the other hand, considers both: the conventional prognostic factors and the signature, and therefore, it will be able to address this question.

In addition, when the data will be ready, we should consider that the intermediate RS spans between 18 and 31, the new results of TAILORx trial will not be totally comparable with the previous data, and moreover, retrospective data refer only to some of the patients enrolled in the different studies, and in most cases analyses have been performed on FPE archival materials. Consequently, the results may be biased in terms of patient selection and pre-analytical variables. Thus far, Oncotype DX® has been done in a single laboratory, which constitutes both the power and the weakness of the test. In fact, the problems of reproducibility have obviously been overcome, but in case of the widespread use of the test in the clinical setting, a single-center assessment is not long possible, and extending the test to peripheral laboratories may lead to a high variability in results.

Therefore, the clinical utility of Oncotype DX® is questionable. Indeed, one-third of patients who have a high risk score would receive chemotherapy also without the results of the test, and, on the other hand, the estimate of risk is widely overlapping in the remaining three-thirds of patients who have an intermediate or low score,[14] and the benefit of chemotherapy is not sufficient to warrant this adjuvant treatment.

Question 3: Can we use genomic predictors to choose the type of chemotherapy?

There are several limits to the use of genomic predictors to select the type of chemotherapy, primarily because the treatment

result depends not only on the (molecular) characteristics of the tumor, but also on the characteristics of the host, which can influence both the pharmacokinetics and the pharmacodynamics of the drug used. Studies exploring the possibility to predict the type of response to a particular drug have been conducted mainly in the neoadjuvant setting. A multigene signature predictive of the activity of paclitaxel and 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC), evaluated based on complete pathological response, has been identified,[71] which was a more powerful predictor of treatment outcome than classical parameters. Unfortunately, its positive predictive value is modest (52%), whereas the negative predictive value is high (92%), that is: we can probably select what not to use, but not what to use.

Question 4: Are genomic predictors ready for routine clinical practice?

Gene-expression profiling clearly has great potential to improve breast cancer management, although the clinical value of gene signatures awaits the results of the ongoing prospective trials of the Oncotype DX® and the Amsterdam signatures (MammaPrint®), which will provide level I evidence about the relevance of applying gene-expression predictors to the daily management of breast cancer patients.

The guidelines also state that the present data are insufficient to recommend use of other assays. It appears however, that the true clinical relevance of these tests needs additional studies to be determined. Two ongoing clinical trials may bring some needed answers:

- *The TAILORx (Trial Assigning Individualized Options for Treatment) Trial*. This prospective clinical trial is sponsored by the National Cancer Institute and involves 900 sites in North America.[72] It will use Oncotype DX to guide treatment selection. This trial plans to enroll at least 10,000 women with ER or PR-positive HER2-negative, lymph node-negative breast cancer. Patients with low Recurrence Score will receive hormonal therapy alone, patients with high Recurrence Score will receive hormonal therapy and chemotherapy, and patients with intermediate Recurrence Score will be randomized into either hormonal therapy alone or hormonal therapy plus chemotherapy. Results from this trial will not appear before 2013.
- *The MINDACT (Microarray in Node-Negative Disease May Avoid Chemotherapy) Trial*. This prospective clinical trial is sponsored by the European Organization for Research and Treatment of Cancer and opened in August of 2007.[73] It plans to compare the 70-gene MammaPrint assay against the standard clinicopathologic prognostic factors included in Adjuvant Online, in selecting 6000 node-negative breast cancer patients for adjuvant chemotherapy. Preliminary results from MINDACT may be presented in 2013. Recently the results of the pilot phase consisting of first 800 patients included were presented: during the pilot phase 46% of screened patients were enrolled. Main reasons for non-enrollment were node positivity before trial amendment, sample quality problems and failure to meet logistic settings. However the proportion of discordant patients, the potential reduction in chemotherapy based treatment by using the genomic signature and compliance to treatment assignment are in accordance with the trial hypotheses.[74]

Various research groups have identified gene expression signatures that predict clinical outcome. Interestingly, although all signatures address the same clinical question, namely, how to

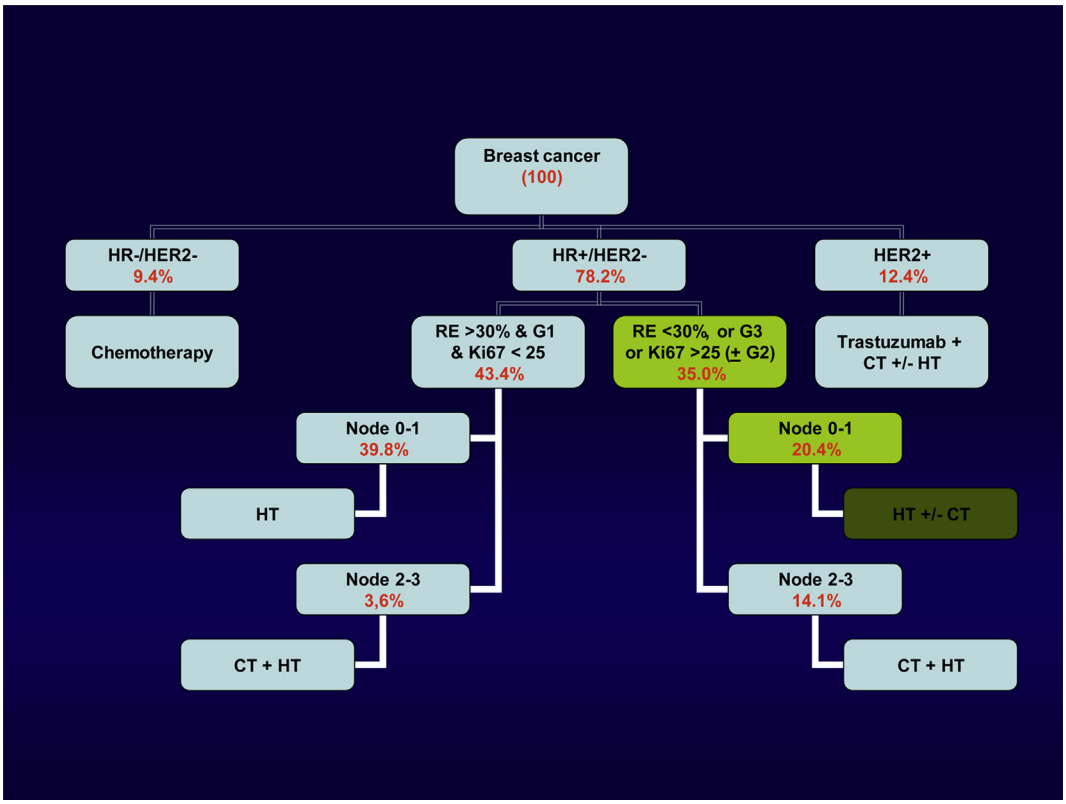

Fig. 1. Decision tree simulation based on clinical and biological variables in 100 patients with early breast cancer. HR: hormone receptor; HT: hormone therapy; CT: chemotherapy; RE: receptor for estrogen; G: grading.

identify a higher proportion of low-risk patients not necessarily needing systemic adjuvant treatment, it is surprising that there is only a small or no overlap between their gene lists, thereby raising questions about their biological meaning. Moreover, in addition to being a clinically heterogeneous disease, breast cancer is also molecularly heterogeneous, with subgroups primarily defined by ER and HER2 expression. The various prognostic signatures available have never been specifically evaluated and compared in these different molecular subgroups. This is probably due to the relatively small sizes of the individual studies, which would have made these findings statistically unreliable. With a view to integrating clinicopathologic and gene expression data, Desmedt et al.[47] recently conducted a comprehensive meta-analysis focusing on the main molecular subtypes of breast cancer: the ER-positive and HER2-negative subgroup, the ER-negative/HER2-negative subgroup and the HER2-positive subgroup. Gene expression modules related to key biological processes in breast cancer such as tumor invasion, immune response, angiogenesis, apoptosis, proliferation, and ER and HER2 signaling were analyzed together with clinical variables and several prognostic signatures on publicly available microarray studies (>2100 patients). In the untreated population, the prognostic impact of proliferation genes is limited to the ER-positive HER2-negative subset since the HER2-positive or ER-negative HER2-negative subsets are associated with high proliferation activity. Therefore, these gene expression-based tests are mainly useful for the ER-positive HER2-negative subset of patients. Since these patients are usually treated with adjuvant antiestrogen therapies, the interaction between the gene expression markers and chemotherapy should be verified in an anti-estrogen-treated cohort in a randomized clinical trial. Although Oncotype Dx is the only test supported by the results of a randomized clinical trial of adjuvant chemotherapy, other gene expression-based tests are expected to provide similar information in prospective studies. Being more reproducible and precise surrogates of tumor grade, gene expression profiling assays (MapQuant Dx and Theros Breast Cancer Index) are very promising tests. However, the absence of a definitive predefined cut-off that defines the subset of patients that benefit from chemotherapy limits their clinical application.

To date, no signature can replace the classical parameters tumor size, nodal status, grade, proliferative activity, ER and HER2 status. In summary, we have new, exciting tools with which to characterize breast tumors and to estimate their behavior although, as yet, these tests cannot be considered a guide for treatment decision. In fact, if we consider 100 patients with early breast cancer surgically treated, we could build a decision tree model based on the “classical” parameter, as reported in Fig. 1. Only in 20 patients, would there probably be a doubt regarding the prescription of chemotherapy. So far, the simple IHC determination of 4 biomarkers (ER, PgR, HER2 and Ki67) is a surrogate for gene signature, and it has several advantages, i.e., low cost, the ready availability of reagents, the relation with cancer cell morphology and the possibility of testing on paraffinized samples. This panel can give both prognostic and predictive information and should be used in all patients, but efforts to improve its standardization and reproducibility are strongly recommended.

Conclusion

Molecular medicine is exploiting pattern-based diagnostic discoveries in genomics and proteomics, with the ultimate aim of discovering new types of biomarkers/biomarker sets, that have improved sensitivity and specificity, for targeted therapies. The challenge is to evaluate the relative contributions of multiple levels of data, both molecular and clinical, in predicting breast cancer outcome and response to anticancer agents. Developing integrative models that combine clinical and complex multilevel molecular factors, such as gene expression patterns, functional proteomics, traditional clinico-pathological risk factors and treatment information, will also increase our understanding of the complex genotype–phenotype inter-relationships involved in breast cancer. Combining one or more gene-expression classifiers into a single model together with traditional clinico-pathological parameters that still retain important prognostic information will probably give us a more comprehensive level of understanding of tumor biology and provide, if prospectively clinically validated, novel and reliable prognostic and predictive factors that will improve the daily management of breast cancer patients.

Conflict of interest statement

None declared.

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