Current Clinical Role of Genetic Profiling in Breast Cancer

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Abstract

Genetic profiling of breast cancer is emerging as an important prognostic and predictive tool, especially for patients with early-stage breast cancer. Several genetic profile assays are already commercially available, and others are being developed and tested. OncotypeDx, a 21-gene assay, and MammaPrint, a 70-gene assay are the most extensively evaluated tests. Currently, three prospective trials to assess the predictive value of gene signature assays in certain subgroups of breast cancer are ongoing. These are the Trial Assigning Individualized Options for Treatment (Rx) (TAILORx) trial, the endocrine-responsive breast cancer (RxPONDER) trial for 21-gene recurrence score and Microarray In Node-negative Disease may Avoid ChemoTherapy (MINDACT) trial using the 70-gene signature.

1 Introduction

Recent research has shown that breast cancer is a heterogeneous disease at the genetic level. The variations in gene expression affect the clinical behavior and course of the disease. In one of the initial studies, Perou and colleagues analyzed the gene expression patterns of 65 breast cancer specimens from 42 individuals (Perou et al. 2000). They found that the tumors showed a wide variation in the patterns of gene expression. However, the patterns from two tumor samples from the same individual were more similar to each other than to any other sample. Based on their findings, they were able to identify what are now known as the biological subtypes of breast cancer: estrogen receptor (ER)-positive, luminal A and B, basal-like, Erb-B2+, normal, and claudin-low. These subtypes of breast cancer have different natural histories and survival patterns, as well as different patterns of response to therapy. Gene expression profiling is not yet routinely performed in the analysis of a breast tumor, so identification of these subtypes is not currently being utilized

clinically. Instead, tumors are now classified clinically based on their ER, progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) status. For practical purposes, the subtypes can be approximated using this clinical data, although there is no perfect correlation between the results of gene expression array tests.

Over the last two decades, significant improvements have been made in breast cancer mortality rates. The 5-year relative survival rate for women with breast cancer increased from 63 % in the early 1960s to 90 % today (American Cancer Society 2011). This improvement has likely been due to earlier diagnosis and improvements in therapy, among other reasons. Adjuvant therapy has been shown to significantly improve disease-free and overall survival in both premenopausal and postmenopausal patients up to 70 years of age with node-negative or node-positive breast cancer. Given the multitude of currently available medical treatments for breast cancer, it is a challenge to select the appropriate adjuvant therapy for an individual patient. Genomic assays have recently become powerful tools in predicting recurrence and mortality, which allows the refinement of therapeutic approaches.

Unlike conventional clinical prognostic factors, genetic profiling has not been validated in prospective randomized clinical trials. Though the results of these tests are reproducible, they are expensive and have limited availability. Also, there is a paucity of data comparing the available tests. Nevertheless, they have the potential to improve and individualize clinical decision making. Until now, decisions regarding adjuvant chemotherapy have been determined by factors including age, performance status, tumor size, tumor grade, tumor stage, lymph node involvement, and ER, PR, and HER2 status. These factors are combined in guidelines such as the National Institutes of Health (NIH) Consensus Development criteria (National Institutes of Health 2000; (Eifel et al. 2001), the St. Gallen expert opinion criteria (Goldhirsch et al. 2001, 2005), the National Comprehensive Cancer Network Guidelines (2011), and a Web-based algorithm, Adjuvant! Online (Ravdin et al. 2001; Adjuvant Inc. 2012). These guidelines suggest that the majority of women with node-negative, estrogen receptor-positive breast cancer should be offered chemotherapy. However, this approach will likely result in overtreatment in many women since only a minority of these patients will develop recurrent disease (Eifel et al. 2001; NCCN 2011). It is generally thought that patients with poor prognostic features benefit the most from adjuvant therapy. For example, axillary nodal involvement has been considered one of the most important prognostic features, with an increasing number of axillary nodes correlating with a more unfavorable clinical outcome (Carter et al. 1989; Page 1991; Rosen et al. 1989). It is thought that patients with positive nodes are most likely to benefit from adjuvant chemotherapy, with an absolute benefit of 6–15 % at 5 years (Early Breast Cancer Trialists' Collaborative Group 2005). However, this population benefit is not true for all individuals, since 25–30 % of node-positive patients will remain free of distant metastases even without systemic adjuvant therapy (Joensuu et al. 1998). Therefore, recommending adjuvant chemotherapy based on the nodal status alone results in overtreatment of a significant portion of patients. If more reliable tests could identify which high-risk patients would benefit from adjuvant chemotherapy, many other patients could be spared from the unnecessary toxicities.

This chapter will focus on the different types of gene expression profiling tests that are available for clinical use in breast cancer and on new tests that are still being developed.

2 Gene Expression Profiling

The level of gene expression reflects the activity of a particular gene. The transcription of genetic DNA into messenger RNA (mRNA) is the first step in gene expression. Technologies such as DNA microarray analysis and real-time reverse-transcriptase polymerase chain reaction (RT-PCR) allow the simultaneous measurement of multiple gene transcriptions. The two FDA-approved tests that are now available in the clinical setting are the 21-gene RT-PCR assay (Oncotype Dx) and the 70-gene signature (MammaPrint). Others that will be described are a two-gene signature (HOX13:IL17BR ratio), Mammostrat, the Rotterdam 76-gene signature, 11-gene EP score, 97-gene genomic grade index, Breast Cancer Index, and the wound-response gene expression.

3 21 Gene RT-PCR Assay (Oncotype DX)

Oncotype DX is a 21-gene assay that measures the expression of 16 tumor-related and 5 reference genes by RT-PCR (reverse-transcriptase polymerase chain reaction). It can be performed on formalin-fixed, paraffin-embedded tissue samples. The 16 tumor-related genes were prospectively chosen from a 250-candidate gene set, selected from an extensive literature review and analyzed for expression and relation to relapse-free survival (RFS) across 3 independent studies of 447 patients, which demonstrated a consistent statistical link between these genes and distant breast cancer recurrence (Paik et al. 2003; Cobleigh et al. 2005; Esteban et al. 2003). Five of the genes are in the proliferation group (Ki-67, STK15, Survivin, Cyclin B1, and MYBL2), two in the HER2 group (HER2 and GRB7), four in the estrogen receptor group (ER, PR, Bcl2, and Scube2), two in the invasion group (Stromelysin3 and Cathepsin L2), and 3 unaligned (macrophage marker CD68, anti-apoptosis gene BAG1, GSTM1). Some of the genes were already well described in the breast

cancer literature, while others were relatively new. Based on these 21 genes, an algorithm was developed to determine a RS, which is divided into low-risk (<18), intermediate-risk (18–30), and high-risk (≥31) categories (Fig. 1).

The 21-gene RT-PCR assay has both prognostic and predictive values. It estimates the likelihood of disease recurrence within 10 years, and it predicts the benefit of chemotherapy and tamoxifen in reducing the risk of recurrence. The use of this test is endorsed by the American Society of Clinical Oncology for women with ER-positive, lymph node-negative, early-stage breast cancer (Harris et al. 2007), the NCCN guidelines 2011, and the St. Gallen International Expert Consensus (Goldhirsch et al. 2009). According to the NCCN guidelines, the 21-gene RT-PCR assay should be considered in determining the need for adjuvant chemotherapy for patients with hormone receptor-positive, HER2-negative tumors that are pT1b-pT3 and N0 or N1mi (≤2 mm axillary nodal metastases). If the RS is low risk (<18), adjuvant endocrine therapy alone is recommended. If the RS is intermediate risk (18–31), chemotherapy should be considered, and if it is high risk (≥31), chemotherapy is recommended.

The 21-gene RT-PCR assay was retrospectively validated in the National Surgical Adjuvant Breast and Bowel Project Trial (NSABP) B-14 (Paik et al. 2004). The original trial prospectively randomized 2,828 node-negative, ER-positive women to receive tamoxifen or placebo, and an additional 1,235 patients were registered to tamoxifen in the 10-month period following closure of the trial in 1988, resulting in a total of 2,617 eligible tamoxifen-treated patients (Fisher et al. 1996, 1999, 2001a, b). RT-PCR was successfully performed in 668 of 675 available tumor blocks. Fifty-one percent of the patients were classified as low risk, 22 % were intermediate risk, and 27 % were high risk. One primary objective was to determine whether the proportion of patients who were free of disease for more than 10 years was significantly greater in the low-risk group than in the high-risk group. The 10-year disease-free survival was 93.2 % for patients in the low-risk group as compared to 69.5 % in the high-risk group, p < 0.001. The RS also provided significant predictive power that was independent of age and tumor size in a multivariate analysis (p < 0.001) (Fig. 2 and Table 1).

The 21-gene RT-PCR assay was further validated in a large population-based, case–controlled study of node-negative, ER-positive patients who were not treated with adjuvant chemotherapy (Habel et al. 2006). Of 4,694 patients diagnosed with invasive breast cancer between 1985 and 1994, a blinded analysis was performed on the tissue of 220 women who had died from breast cancer and 570 women who had not. The RS correlated with the risk of breast cancer
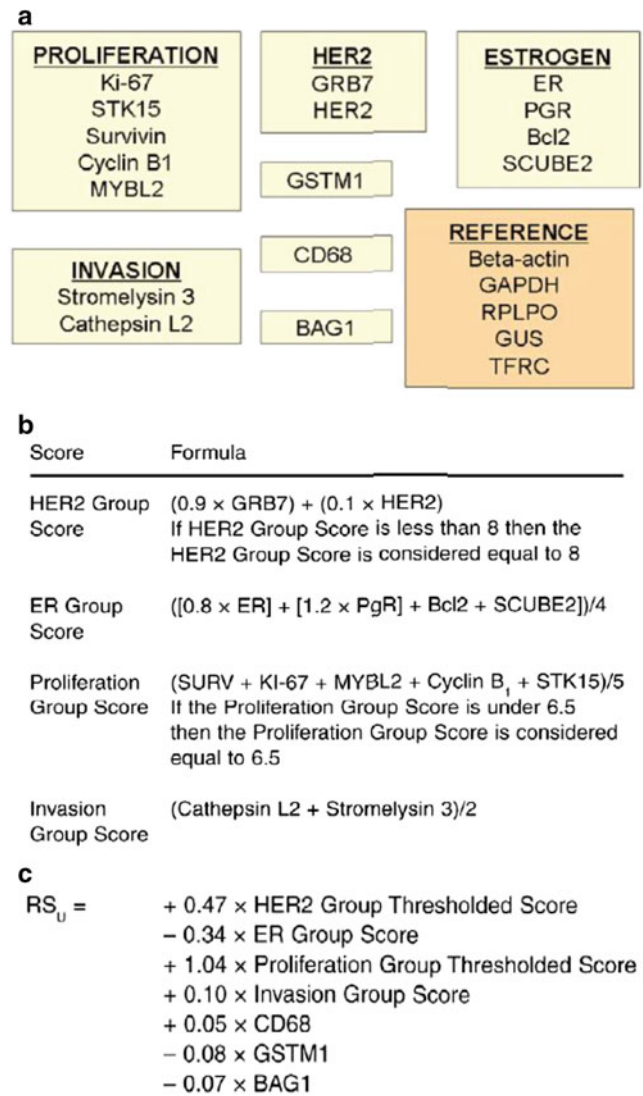

Fig. 1 a The final gene list (16 cancer-related and five reference genes) and summary score (recurrence score) algorithm for this assay were developed by analyzing the results of three independent preliminary breast cancer studies (training sets) with a total of 447 patients. The recurrence score, on a scale from 0 to 100, is derived from the reference-normalized expression measurements in four steps. In the first step, the expression for each gene is normalized relative to the expression of the five reference genes (b-actin, GAPDH, GUS, RPLPO, and TFRC). Reference-normalized expression measurements range from 0 to 15, where a 1-unit increase reflects approximately a twofold increase in RNA. b In the second step, the HER2 group score, the ER group score, the proliferation group score, and the invasion group score are calculated from individual gene expression measurements. c In the third step, the recurrence score unscaled (RSu) is calculated using coefficients that were predefined based on regression analysis of gene expression and recurrence in the three training studies (providence, rush, and NSABP B-20). A plus sign indicates that increased expression is associated with recurrence risk. A minus sign indicates that increased expression is associated with decreased recurrence risk. Source Habel et al. 2006

Fig. 2 Likelihood of distance recurrence, according to recurrence score categories. A low risk was defined as a recurrence score of less than 18, an intermediate risk as a score of 18 or higher but less than 31, and a high risk as a score of 31 or higher. There were 28 recurrences in the low-risk groups, 25 in the intermediate-risk group, and 56 in the high-risk group. The difference between the groups is significant (P <0.001). *Source* Paik et al. 2004
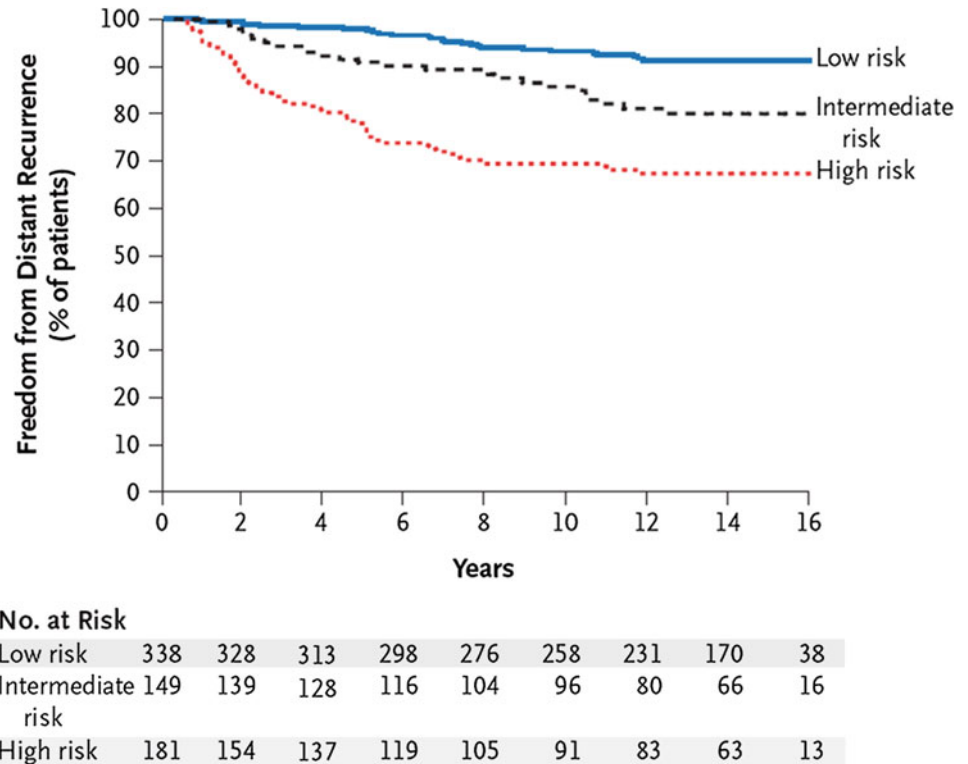

Table 1 Kaplan–Meier estimates of the rate of distant recurrence at 10 years, according to recurrence score-risk categories

Risk category	Percentage of patients	Rate of distant recurrence at 10 yr (95 % confidence interval) (%)
Low	51	6.8 (4.0–9.6)
Intermediate	22	14.3 (8.3–20.3)
High	27	30.5 (23.6–37.4)*

*$P < 0.001$ for the comparison with the low-risk category (*Source* Paik et al. 2004)

death in this population, after adjusting for tumor size and grade, in both tamoxifen-treated and tamoxifen-untreated patients ($P = 0.003$ and $P = 0.03$, respectively). The RS provided information independent of tumor size and grade. The relative risk estimations for RS in the ER-positive patients were similar to those in NSABP B-14 (Paik et al. 2004).

In a single smaller analysis, the 21-gene RT-PCR assay did not correlate with recurrence-free survival (Esteva et al. 2005). The RS was performed on archival paraffin-embedded tissue samples of 144 patients with node-negative, invasive breast cancer who received no systemic adjuvant therapy. The RS was not predictive of distant disease recurrence. There was a high concordance between the RT-PCR results and immunohistochemical assays for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 status. When attempting to reconcile the results of this series to others, it is important to note

that in this series alone, ER-negative patients were included. In addition, a high tumor grade was associated with a better prognosis in this study, calling into question the validity of this series.

In addition to its prognostic value, the 21-gene RT-PCR assay has been shown to be predictive of benefit from tamoxifen and chemotherapy. In NSABP B-14, patients treated with tamoxifen were compared to those treated with placebo. The patients with the low- and intermediate-risk RS who received tamoxifen had large improvements in disease-free survival, while those with high-risk RS had a smaller benefit (Paik et al. 2004). NSABP B-20 was a phase III trial that randomized 2,363 patients to receive tamoxifen either alone or tamoxifen with chemotherapy (either cyclophosphamide, methotrexate, and fluorouracil or methotrexate and fluorouracil) (Paik et al. 2006). RT-PCR was successfully performed in 651 patients (227 randomized to tamoxifen, 424 randomized to tamoxifen plus chemotherapy). The distribution of age, tumor size, tumor grade, and hormone receptor status was similar to the entire trial population. In this group, 54.2 % of the patients were low-risk, 20.6 % intermediate-risk, and 25.2 % high-risk RS. For the low-risk patients, the addition of chemotherapy added no benefit in reducing the risk of distant recurrence at 10 years (relative risk, 1.31; 95 % CI, 0.46–3.78; increase of 1.1 % in absolute risk), while there was a large reduction in distant recurrence at 10 years for the high-risk category (relative risk, 0.26; 95 % CI, 0.13–0.53; decrease of 27.6 % in absolute risk).

The benefit from chemotherapy was less clear for patients in the intermediate-risk group (relative risk, 0.61; 95 % CI, 0.24–1.59; 1.8 % increase in absolute risk). Given the uncertainty in the estimate, a clinically important benefit could not be excluded for the intermediate-risk patients.

The value of the RS in predicting response to neoadjuvant chemotherapy in locally advanced breast cancer has been confirmed in two studies. In one study, 89 patients were treated with neoadjuvant doxorubicin and paclitaxel, and 11 (12 %) had a complete pathologic response (pCR) (Gianni et al. 2005). The RS was positively associated with the likelihood of pCR ($p = 0.005$), suggesting that patients who had the greatest risk of distant recurrence are likely to derive the greatest benefit from chemotherapy. In the second study, 97 patients had core biopsies taken prior to treatment with neoadjuvant docetaxel (Chang et al. 2008). Eighty (82 %) of the specimens had sufficient RNA for RT-PCR, and in 72 (74 %) of the patients, clinical response data were available. Clinical complete responses were more likely in the high-RS group ($p = 0.008$). Tumors with significant increases in the proliferative gene group and decreases in the ER gene group were most likely to respond to chemotherapy.

The 21-gene RT-PCR assay was evaluated in a more contemporary population of women with early-stage, hormone receptor-positive, node-negative and node-positive, operable breast cancer in an analysis of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial (Dowsett et al. 2010). In this trial, postmenopausal women were randomized to anastrozole, tamoxifen, or both drugs. Among the 4,160 patients in the monotherapy arms, there were 1,231 evaluable patients in whom the RS was determined; 71 % were node-negative, 25 % were node-positive, and 4 % had unknown nodal status. In both node-negative and node-positive patients, the RS was significantly associated with time to distant recurrence by multivariate analyses ($p < 0.001$ and $p = 0.002$, respectively). The RS also showed significant prognostic value beyond that provided by adjuvant! online ($p < 0.001$). In node-negative patients, 9-year distant recurrence (DR) rates in low (RS < 18), intermediate (RS 18–30), and high-RS (RS $\geq$ 31) groups were 4, 12, and 25 %, respectively, and 17, 28, and 49 %, respectively, in node-positive patients. This study validated the RS in the tamoxifen-treated population. In this analysis, the relative risk reduction was similar across the different RS groups. Overall, the ATAC trial demonstrated a 16 % relative reduction in the rate of distant recurrence for patients treated with anastrozole. This analysis established that the relationship between the RS and DR could be applied to patients treated with anastrozole, with an approximate 16 % adjustment for the lower risk of distant recurrence for those patients. Also, this study confirmed the poor correlation between the RS and adjuvant! online although both measures provided substantial independent prognostic information.

The 21-gene RT-PCR assay is currently recommended for use in women with node-negative, hormone receptor-positive tumors, although some of the original work was done in node-positive patients (Cobleigh et al. 2005). In one study, RNA was extracted from 78 paraffin tumor blocks of patients with breast cancer diagnosed between 1979 and 1999. All of the patients had ten or more lymph nodes involved (median 15 lymph nodes). At the time of publication, 77 % of the patients had distant disease recurrence or breast cancer-related death. When the RS was obtained, 11 patients (14 %) had a RS < 18 with a rate of distant recurrence at 10 years of 29 %, 19 patients (24 %) had a RS of 18–31 with a rate of distant recurrence at 10 years of 72 %, and 48 patients (62 %) had a RS of $\geq$ 31 with a rate of distant recurrence at 10 years of 80 %. This showed that there was a subset of node-positive patients with a low RS who had a prolonged disease-free survival.

Recently, the RS has been tested retrospectively in a randomized trial of node-positive women, Southwest Oncology Group (SWOG) 8814 (Albain et al. 2010). The original trial showed that chemotherapy with CAF (cyclophosphamide, doxorubicin and 5-fluorouracil) given before tamoxifen improved disease-free and overall survival when compared to tamoxifen alone in postmenopausal women with node-positive, ER-positive breast cancer. The two primary objectives of the retrospective analysis were to determine whether the RS assay could provide prognostic information for women with node-positive disease treated with tamoxifen alone and whether the assay could identify a subset of node-positive patients who did not benefit from the addition of chemotherapy. An analysis was performed on 367 specimens from the original trial (40 % of the patients in the CAF-T and T-alone groups). This subset of patients resembled the patients in the original study except for a slightly lower number of positive lymph nodes and smaller tumor size. When adjusted for the number of positive lymph nodes, the benefit in disease-free and overall survival was similar for CAF-T over T alone, as was seen in the parent trial. The RS was highly prognostic in the T-alone group, with 10-year DFS estimates of 60, 49, and 43 % for low-, intermediate-, and high-risk categories. The RS was also a strong predictive factor of benefit from CAF chemotherapy. There was no benefit for chemotherapy in women who had a low-risk RS (stratified log-rank $p = 0.97$; HR 1.02, 95 % CI 0.54–1.93), whereas those with a high-risk RS had a significant improvement in disease-free survival (stratified log-rank $p = 0.033$; HR 0.59, 0.35–1.01). This analysis suggests that there may be subsets of women with ER-positive, node-positive disease who do not derive additional benefit from adjuvant anthracycline-based chemotherapy.

The 21-gene RT-PCR assay has been shown to be superior to standard clinical and pathologic factors (Goldstein et al. 2008). In a study of 465 patients with hormone

receptor-positive breast cancer with zero to three positive axillary nodes, the RS was a powerful prognostic factor for recurrence in both node-negative and node-positive disease (p < 0.001 for both). It was more strongly associated with recurrence than clinical variables, which were integrated by an algorithm modeled after adjuvant! that was adjusted to 5-year outcomes. The 5-year recurrence rate was only 5 % or less for the estimated 46 % of patients who have a low RS (<18).

The prognostic utility of the RS and adjuvant! was compared in the 668 tamoxifen-treated patients in NSABP B-14, 227 tamoxifen-treated patients in NSABP B-20 and 424 chemotherapy and tamoxifen-treated patients in NSABP B-20 (Tang et al. 2011). Adjuvant! uses patient and tumor characteristics to predict the clinical outcome, and is routinely used in practice (Ravdin et al. 2001; Olivotto et al. 2005). Adjuvant! also utilizes the results of the Early Breast Cancer Clinical Trialists' Collaborative Group (EBCTCG) overview to assign benefit from adjuvant therapies, assuming the same proportional reduction in recurrence and mortality across different prognostic categories (EBCTCG 2005). The results showed that the RS and adjuvant! were independently prognostic for the risk of distant recurrence. In the NSABP B-20 cohort with RS results available, the RS was significantly predictive of chemotherapy benefit (interaction $p = 0.031$ for DRFI, $p = 0.011$ for OS), whereas adjuvant! was not significantly predictive (interaction $p = 0.99$ and $p = 0.311$, respectively).

The 21-gene RT-PCR can reclassify patients who were considered high risk by conventional prognostic markers to a low-risk group. Paik et al. (2005) showed that the 21-gene RT-PCR assay was more accurately predictive than the St. Gallen or National Comprehensive Cancer Network risk stratification guidelines, and this could be used to change some patient decisions about chemotherapy. In this study, about half of the patients who were in the high-risk category as defined by the NCCN guidelines could be reclassified as low-risk by the 21-gene RT-PCR assay, with a 10-year relapse risk of 7 % (CI, 4–11 %). This is similar to the relapse rate seen in the low-risk RS group without the NCCN information. A separate study also compared RS with adjuvant! In the 668 tamoxifen-treated patients from NSABP-B14 (Bryant 2005), 32 % of the patients were low-risk according to both algorithms. Overall, there is about a 48 % concordance between the RS and adjuvant! online-risk categories. About 18 % of patients are classified as low risk according to one algorithm, but high risk according to the other. The RS correlated more strongly with outcome than did adjuvant! These findings suggest that the greatest impact of the RS is in reclassifying patients from high to low risk, thereby reducing the number of women who would be given chemotherapy unnecessarily.

Recently, evidence has emerged that standard immuno-histochemical markers can have a predictive value similar to the RS. In a study of 1,125 patients from the ATAC trial, a comparison of the Oncotype DX with the IHC4 score (a formula utilizing four standard immunohistochemical markers: ER, PR, Ki67, and HER2) showed that all four IHC markers provided independent prognostic information in the presence of classical variables (Cuzick et al. 2011). The information from the IHC4 score was similar to that in the RS, and little additional prognostic value was seen in the combined use of both scores. These preliminary results suggest that four standard IHC assays performed in a high quality laboratory can provide prognostic information similar to the RS for endocrine-treated ER-positive breast cancer patients. However, additional studies are required to determine the reproducibility and general applicability of this test.

A formal integration of the RS and the classic pathologic and clinical factors, such as tumor size, tumor grade, and patient age, has been performed and will soon be available online (Tang et al. 2010, 2011). In this meta-analysis, which included 647 patients from NSABP B-14 and 1,088 patients from the ATAC trial, the risk of distant recurrence was assessed by using the RS, pathologic factors, and clinical information. These disparate sources of information were then combined to derive the RS-pathology-clinical (RSPC) assessment of distant recurrence risk. The RSPC model provided significantly improved prognostic results for distant recurrence risk compared with the RS alone ($p < 0.001$), or compared with a model using tumor grade, size, and patient age ($p < 001$). Compared with the RS alone, there was an improved separation of risk, with a 33 % relative reduction in the number of patients with intermediate RS (17.8 % for RSPC vs. 26.7 % for RS, $p < 0.001$) and an 18 % relative increase in the number of patients with a low RS (63.8 % for RSPC vs. 54.2 % for RS, $p < 0.001$). This RSPC model will likely have its greatest utility in these low- and intermediate-risk patients.

An association has been demonstrated between the RS and the risk of locoregional recurrence (LRR) (Mamounas et al. 2010). The study analyzed 895 tamoxifen-treated patients from the NSABP B-14 and B-20 trials, 355 placebo-treated patients from B-14, and 424 chemotherapy and tamoxifen-treated patients from B-20. The primary endpoint was the time to first LRR. In the tamoxifen-treated patients, the risk of LRR was significantly correlated with the RS-risk groups ($p < 0.001$). The 10-year estimate of LRR was 4.3 % for the low-risk, 7.2 % for the intermediate-risk, and 15.8 % for the high-risk RS groups. There was also a significant association between LRR and the RS in the placebo-treated group ($p = 0.022$) and the chemotherapy and tamoxifen-treated group ($p = 0.028$). These results are not surprising

given the strong associations between LRR and distant recurrence, and they may be helpful in making clinical decisions regarding locoregional therapy.

The use of the 21-gene RS assay can have an impact on both physician and patient decisions about adjuvant therapy. A multicenter study was conducted to prospectively determine whether the RS affects physician and patient adjuvant treatment selection and satisfaction (Lo et al. 2010). Physician adjuvant treatment recommendations were assessed before and after obtaining the RS in 89 assessable patients. Patients were also asked about their treatment choices before and after the RS was obtained, and measures of decisional conflict, anxiety and quality of life were assessed. In 28 patients (31.5 %), the recommendation of the medical oncologist was changed when the RS score was provided. The largest change was from a pretest recommendation of chemotherapy plus hormonal therapy to a post-test recommendation of hormonal therapy only. This occurred in 20 patients (22.5 %). Nine patients (10.1 %) changed their treatment decision from chemotherapy and hormonal therapy to hormonal therapy only. Medical oncologists reported an increased confidence in their treatments in 68 cases (76 %). Patient anxiety and decisional conflict were significantly lower after RS results were provided.

Similar results have been shown across six other independent decision impact studies (Asad et al. 2008; Henry et al. 2009; Klang et al. 2010; Liang et al. 2007; Oratz et al. 2007; Thanasoulis et al. 2008). A meta-analysis of these studies included a total of 912 patient from both academic and community centers in the United States and showed that there was a consistently large impact of the RS on treatment decisions in both directions (Hornberger and Chien 2010, 2011). Overall, the RS led to a 37 % change in treatment decisions. In 52 % of patients, there was a switch from the initial recommendation of chemotherapy and hormonal therapy to hormonal therapy alone and in 12 % of patients, there was a switch from the initial recommendation of hormonal therapy alone to chemotherapy and hormonal therapy. Results from this meta-analysis underscore a consistent and large impact of the RS on treatment decisions by physicians. Recommendations changed in more than a third of treatment decisions after integrating the RS information with traditional measures.

In addition to RS, Genomic Health also includes the results of ER, PR, and HER2 testing by RT-PCR assessment in their reports. A study of 776 breast cancer patients from the Eastern Cooperative Oncology Group (ECOG) E2197 compared ER and PR measured by local laboratory immunohistochemistry (IHC), central IHC, and central reverse-transcriptase polymerase chain reaction (RT-PCR) using the 21-gene assay. There was a high degree of concordance between the three assays (84–93 %) (Badve et al. 2008).

Although ER expression was marginally associated with relapse in ER-positive patients treated with chemotherapy and hormonal therapy, the RS was a highly significant predictor of recurrence in these patients. Despite this excellent concordance, evidence showing the prognostic and predictive value of the qRT-PCR cutoffs to define positivity is still awaited. A study comparing central laboratory HER2 testing by fluorescence in situ hybridization (FISH) to RT-PCR in lymph node-negative, chemotherapy-untreated patients from a large Kaiser Permanente case–control study showed that HER2 concordance by central FISH and central RT-PCR was 97 % (95 % CI, 96–99 %) (Baehner et al. 2010). In contrast, in an independent quality assurance study of 843 patient cases comparing local FISH testing for HER2 to available HER2 RT-PCR results from Genomic Health, there was an high false-negative rate for HER2 status with the RT-PCR assay (Dabbs et al. 2011). Therefore, RT-PCR-based assessments of ER, PR and HER2 should be interpreted together with the results of the FDA-approved methods for assessment of these biomarkers.

The role of gene expression profiling in the treatment of ductal carcinoma in situ (DCIS) has recently been evaluated. A new, prespecified DCIS Score was analyzed to predict recurrence in patients from the ECOG 5194 trial (Solin et al. 2011). In that trial, 670 eligible patients with low- or intermediate-grade DCIS ≤ 2.5 cm or high-grade DCIS ≤ 1 cm were treated with surgical excision only, without radiation, and 228 received tamoxifen (Hughes et al. 2009). RT-PCR analysis was performed in 327 patients (49 % of the original population). The primary objective was to determine whether there was a significant association between the risk of an ipsilateral breast event (IBE) and the continuous DCIS Score. With a median follow-up of 8.8 years, the study was able to prospectively validate that the DCIS score quantifies recurrence risk and complements traditional clinical and pathologic factors.

Prospective clinical trials to evaluate the 21-gene RT-PCR assay are ongoing. The TAILORx trial has completed accrual, but the results have not yet been reported. This is the largest randomized adjuvant trial ever conducted, enrolling over 10,000 patients. All of the patients had the 21-gene RT-PCR assay performed, and those with a RS between 11 and 25 were randomized to either hormonal therapy alone or hormonal therapy with chemotherapy. Patients with a RS ≤ 10 were treated with hormonal therapy only and those with a RS > 25 were given chemotherapy and hormonal therapy. The RS ranges for this trial have been altered from the original definitions of low, intermediate, and high risk to minimize potential for undertreatment in the high- and intermediate-risk groups. Another trial, the RxPONDER trial, also known as SWOG S1007, was opened in January 2011 and is currently accruing patients. The study will

randomize 4,000 patients with early-stage, hormone receptor-positive, HER2-negative breast cancer with 1–3 positive lymph nodes who have an RS of ≤ 25 to receive either chemotherapy plus endocrine therapy or endocrine therapy alone. Patients will be stratified into groups by RS 0–13 versus 14–25, by menopausal status, and by axillary lymph node dissection versus sentinel lymph node biopsy. Results from both of these trials will help to further validate the RS and to more clearly define the role of the 21-gene RT-PCR assay in the node-positive population.

4   70 Gene Signature (MammaPrint)

The 70-gene signature (MammaPrint) is a purely prognostic assay for women less than 61 years of age with node-negative, ER-positive, or ER-negative breast cancer. Outside of the United States, it is also being used for patients with 1–3 positive nodes. This test uses DNA microarray technology to determine gene expression, using fresh frozen tumor samples. It can also be performed on formalin-fixed, paraffin-embedded tissue, although the data validating this technique are limited.

The assay focuses on genes involved in proliferation and also measures genes regulating invasion, metastases, stromal integrity, and angiogenesis. It does not directly assess ER, PR, or HER2. The test gives dichotomous results, predicting either a high or low risk of disease recurrence. A correlation coefficient is calculated between a patient's expression levels of the 70 genes and an average good-prognosis expression profile. If the correlation coefficient exceeds 0.4, the patient is classified as having a good-prognosis signature, whereas a coefficient less than 0.4 is classified as a poor-prognosis signature.

In 2007, the 70-gene signature test received approval by the FDA as a prognostic test for breast cancer patients less than 61 years, with tumors less than 5 cm, node-negative and stage I or II breast cancer (Harris et al. 2007). It is approved for both ER-positive and ER-negative disease, but its use in ER-negative disease is limited by the fact that less than 10 % of those tumors will have a good-prognosis signature. The American Society of Clinical Oncology has determined that definitive recommendations for the use of this assay will require data from more clearly directed retrospective studies or from the ongoing MINDACT Trial which will be discussed later.

The 70-gene signature was developed at the Netherlands Cancer Institute, where investigators performed an analysis of gene expression arrays on frozen tissue from 98 sporadic primary breast tumor samples (van't Veer et al. 2002). All of the women were less than 55 years old with tumors less than 5 cm and negative lymph nodes. All of the patients were treated with locoregional therapies only. Seventy-eight

(80 %) were sporadic cases, 18 had BRCA 1 mutations, and two had BRCA 2 mutations. Of the original 78 sporadic tumors, 34 (44 %) had distant metastases within 5 years, whereas 44 patients (66 %) did not. A set of 231 genes was initially identified and found to be statistically significantly associated with disease outcome, defined as the presence of distant metastases within 5 years. This group of genes was then refined to a core group of 70 genes. This 70-gene set had an 83 % accuracy at differentiating patients who developed distant disease relapse from those who did not. The classifier correctly predicted the disease outcome for 65 of the 78 patients (83 %) with 5 poor-prognosis signature patients. Eight good-prognosis signature patients were assigned incorrectly.

The 70-gene signature assay was then validated in several studies. The first trial was a retrospective analysis that included 295 young patients (age <53 at diagnosis) with T1 or T2 tumors (van de Vijver et al. 2002). Of note, 61 of these node-negative patients were also part of the original study done to establish the 70-gene profile, which has been one of the criticisms of this validation study. Of the 295 patients, 151 patients were node-negative and 144 were node-positive; 69 patients were ER-negative and 226 were ER-positive. Adjuvant treatment was given to 10 of the 151 node-negative patients and 120 of the 144 node-positive patients. The treatment consisted of chemotherapy in 90 patients, hormone therapy in 20 patients, and a combination of both in 20 patients. The patients were followed for nearly 7 years. Good-prognosis signatures were seen in 115 patients and poor-prognosis signatures in 180. Patients with node-negative and node-positive diseases were evenly distributed between the two signature groups, indicating that the prognosis profile was independent of the nodal status. There was a strong correlation between the good-prognosis 70-gene signatures and the absence of death or early distant recurrence. Overall 10-year survival rates were 94.5 ± 2.6 % and 54.6 ± 4.4 %, respectively, for the good- and poor-prognosis signature groups. At 10 years, the probability of remaining free of distant metastases was 85.2 ± 4.3 % in the group with a good-prognosis signature and 50.6 ± 4.5 % in the group with a poor-prognosis signature. The odds ratio (OR) for the development of distant metastases at 5 years in the node-negative patients (excluding the patients that overlapped with the prior study) was 15.3, similar to the result of 15 seen in the previous study. For the node-positive patients, the prognostic signature was also highly significant, with an OR of 13.7, $p < 0.001$. In the multivariate analysis, the poor-prognosis signature was the strongest prognostic factor for the development of distant metastases. The prognosis profile was significantly associated with histological grade ($p < 0.001$), ER status ($p < 0.001$), and age ($p < 0.001$) but not with tumor size, extent of vascular invasion, number of lymph nodes involved or the treatment given. This study

also evaluated the node-negative patients after they were divided into risk categories based on clinical-pathological criteria using the St. Gallen criteria (Goldhirsch et al. 2001) and the NIH criteria (Eifel et al. 2001). The gene signature profile assigned more patients to the low-risk or good-prognosis signature groups than traditional methods did: 40 % for the 70-gene assay, 15 % for the St. Gallen criteria, and 17 % for the NIH criteria. The low-risk patients, identified by a good-prognosis signature, had a higher likelihood of metastasis-free survival than those identified as low risk by the St. Gallen or NIH criteria. In addition, the patients identified as high risk by a poor-prognosis signature tended to have a higher rate of distant metastases than did patients identified as high risk by the St. Gallen or NIH criteria. This led to the conclusion that clinical–pathological criteria could misclassify a significant number of patients and could thus result in many patients being either over-treated or under-treated. In this study, the 70-gene signature was the strongest prognostic factor for distant metastasis-free survival, independent of adjuvant treatment, tumor size, lymph node status, histological grade, or age.

A second study was an independent validation of the 70-gene signature in 307 women, less than 60 years of age, with node-negative, T1 or T2 primary tumors who had not been treated with adjuvant systemic therapy (Buyse et al. 2006). The median follow-up was 13.6 years. Frozen samples were available for the 70-gene signature analysis, and the tumors were scored as low or high risk. The tumors were also assigned to clinical risk categories based on adjuvant! online criteria (patient age, comorbidities, tumor size, tumor grade, ER status, and nodal involvement) (Adjuvant!! Inc. 2012). The authors determined that the low-clinical risk group would be defined as patients with a 10-year overall survival probability of at least 88 %, if 10 % or more of the tumor cells expressed detectable ER, or of at least 92 %, if ER expression was seen in less than 10 % of the tumor cells. When adjusted for clinical risk groups based on the 10-year survival probability as calculated by adjuvant!, the 70-gene signature performed independently of clinical variables in predicting time to distant metastases (HR 2.13, 95 % CI 1.19–3.82) and overall survival (HR 2.63, 95 % CI 1.45–4.79), but not disease-free survival. High-risk patients had a 10-year overall survival of 70 % compared to 90 % for those with low-risk signatures. This study showed that the 70-gene signature provides prognostic information independent of the traditional clinical and pathological risk factors in patients with early-stage breast cancer untreated with systemic therapy.

A third validation study evaluated 123 patients less than 55 years of age with T1-2 N0 breast cancer diagnosed between 1996 and 1999, with a median follow-up of 5.8 years (Bueno-de-Mesquita et al. 2009). Adjuvant treatment was given to 45 patients (37 %): 18 (15 %) received chemotherapy, 14 (11 %) received endocrine therapy, and 13 (11 %) received both. Good-prognosis signatures were seen in 52 % and poor-prognosis signatures in 48 % of patients. The poor-prognosis signatures were associated with larger tumors, higher histological grade, and ER-negative and PR-negative status. The 5-year overall survival was 97 ± 2 % for the good-prognosis signatures and 82 ± 5 % for the poor-prognosis signatures, HR 3.4, 95 % CI 1.2–9.6, $p = 0.021$. The 5-year distant metastasis (as first event)-free percentage was 98 ± 2 % for the good-prognosis and 78 ± 6 % for the poor-prognosis signatures, HR 5.7, 95 % CI 1.6–2.0, $p = 0.007$. In a multivariate analysis, the prognosis signature was an independent prognostic factor and outperformed the clinical and pathological criteria.

There are clinical data to suggest that the 70-gene signature can predict the response to chemotherapy, although this has not been sufficiently validated for clinical use. In one study, 167 patients with stage I–III breast cancer were analyzed prior to neoadjuvant chemotherapy, and the rate of pathological complete response (pCR) was used to measure chemosensitivity (Straver et al. 2010). Good-prognosis signatures were seen in 23 patients (14 %) and poor-prognosis signatures in 144 patients (86 %). All 38 of the triple-negative (ER-, PR-, and HER2-negative) patients had poor-prognosis signatures. Pathologic complete responses were seen in 29 of the 144 patients with poor-prognosis signatures and in none of the 23 patients with good-prognosis signatures, $p = 0.015$. The authors concluded that the patients with poor-prognosis signatures were more sensitive to chemotherapy. Two other studies have also shown that patients with poor-prognosis signatures are more likely to achieve an excellent pathological response with neoadjuvant chemotherapy than those tumors expressing a good-prognosis profile (Esserman et al. 2009; Pusztai et al. 2008). Another study showed a significant survival benefit for the addition of adjuvant chemotherapy in patients with the poor-prognosis signature but not for those with a good-prognosis signature (Knauer et al. 2010). In 541 patients, the 70-gene signature classified 252 patients (47 %) as low risk and 289 (53 %) as high risk. Within the low-risk group, there was no significant difference in the 5-year breast cancer-specific survival (BCSS) between patients who received endocrine therapy alone and those who received chemotherapy and endocrine therapy, 97 % versus 99 %, $p = 0.62$. In the high-risk group, the 5-year BCSS was 81 % for those who received endocrine therapy and 94 % for the endocrine therapy and chemotherapy, $p < 0.01$). Similarly, distant disease-free survival (DDFS) at 5 years was not significantly different for endocrine therapy alone or endocrine therapy with chemotherapy for the low-risk group (93 % vs. 99 %, $p = 0.20$), whereas it was significantly better for the high-risk patients with the addition of chemotherapy (76 % vs. 88 %, $p < 0.01$).

The 70-gene signature has been evaluated in other groups of breast cancer patients, including postmenopausal women, and patients with positive lymph nodes. In one study, 148 patients aged 55–70 with T1-2 N0 tumors were analyzed, and 91 (61 %) were found to have good risk, while 57 (39 %) had poor-risk signatures (Mook et al. 2010). In these patients, the BCSS at 5 years was 99 and 80 % for the good and poor-risk groups, respectively (p = 0.036). The distant metastasis-free survival rates were 93 and 72 %, respectively. The 70-gene prognosis signature was a significant and independent predictor of BCSS during the first 5 years of follow-up with an adjusted hazard ratio (HR) of 14.4 (95 % confidence interval 1.7–122.2; P = 0.01) at 5 years. These patients were also analyzed by adjuvant! criteria, which identified 74 patients (50 %) as clinical low risk and 74 patients (50 %) as clinical high risk. There was disagreement with the genomic prognosis in 41 (28 %) patients. Twelve (8 %) patients were identified as clinical low risk but had poor-prognosis genomic signatures, and 29 (28 %) of patients were clinical high risk but had good-prognosis signatures. This study validated the prognostic utility of the 70-gene signature in postmenopausal women and showed that its greatest strength was in the first 5 years after diagnosis. The authors concluded that the beneficial effects of chemotherapy in postmenopausal women occur mostly in the first 5 years after diagnosis and the accurate identification of patients who will have early events, using this signature, may be helpful in selecting patients for adjuvant chemotherapy. A second study retrospectively evaluated 100 postmenopausal patients (median age 62.5) with node-negative disease with the 70-gene signature and adjuvant! online criteria (Wittner et al. 2008) In this study, 27 patients were identified as low risk by the 70-gene signature. None of these patients had distant metastasis as a first event, leading to a negative predictive value of 100 %. Seventy-three patients were identified as high risk by the 70-gene signature. Of these, 9 had distant metastasis as the first event and the other 64 did not. This led to a positive predictive value of 12 %, which was lower than had previously been observed. In comparison with adjuvant! online, the 70-gene signature identified an additional 21 patients as low risk, and none of these patients developed a distant metastasis as the first event.

The 70-gene signature has been shown to have prognostic value in node-positive disease as well. In one of the original validation studies, 144 of the 295 patients had node-positive disease (van de Vijver et al. 2002). Fifty-five of the patients had good-prognosis and 89 had poor-prognosis signatures, and the 70-gene prognostic signature was highly predictive of the risk of distant metastases in these node-positive patients. Although nodal involvement is considered to be predictive of poorer survival, this analysis demonstrated that there is a group of patients who may have a favorable prognosis, despite having positive axillary nodes.

In another study of node-positive patients, 241 patients with T1 to operable T3 tumors with 1–3 positive axillary lymph nodes, including those with micrometastases, were analyzed using the 70-gene signature (Mook et al. 2009). The patients received local treatment followed by adjuvant systemic therapy, according to national guidelines and patient preferences. The 70-gene signature was performed, and clinical risk assessment was also determined by adjuvant! The 70-gene signature classified 99 (41 %) as good prognosis and 142 (59 %) as poor prognosis. The poor-prognosis signature patients were more likely to have received adjuvant chemotherapy, less likely to have received endocrine therapy, and tended to have larger, more poorly differentiated, ER- and PR-negative, and HER2-positive tumors. Breast cancer-specific survival (BCSS) at 5 years was 99 % for the good-prognosis signature vs. 80 % for the poor-prognosis signature group (P = 0.036). The 10-year distant metastasis-free survival (DMFS) and BCSS were 91 and 96 % for the good-prognosis signature group and 76 and 76 % for the poor-prognosis signature group. Using adjuvant! online, 32 patients (13 %) were classified as clinical low risk and 209 (87 %) were classified as clinical high risk. The clinical risk category was discordant with the 70-gene prognosis signature in 77 patients (32 %): 5 patients were identified as clinical low-risk with a poor-prognosis signature, whereas 72 were classified as clinical high-risk with a good-prognosis signature. For the 209 patients identified as clinical high risk, the 10-year BCSS was 94 % for those in the good-prognosis signature group and 76 % for those in the poor-prognosis signature group. In the 27 patients classified as low risk by both the adjuvant! online criteria and the 70-gene signature, none developed distant metastatic disease or died. Again, the 70-gene prognosis signature outperformed traditional prognostic factors in predicting disease outcome in patients in this population and accurately identified some patients with 1–3 positive nodes who had a favorable outcome.

The original 70-gene signature was generated on microarrays that were not designed for processing of many samples on a routine basis. To improve its clinical utility, a customized microarray (marketed as MammaPrint) with a reduced set of probes was developed. One study re-analyzed the 145 patients from the original validation study (van de Vijver et al. 2002) and the 78 patients from the training set (van't Veer et al. 2002), compared the results from the original analysis to the custom microarray, and found an extremely high correlation of prognostic prediction between the two assays (p < 0.0001) (Glas et al. 2006).

Currently, a large prospective randomized trial, the MINDACT, is being conducted in Europe. This study compares the 70-gene signature to the traditional clinical and pathological criteria used to select patients for adjuvant chemotherapy. The trial opened in February 2007, and the

plan is to enroll 6,000 early-stage breast cancer patients (T1, T2, and operable T3M0). Originally, the study included only node-negative patients, but more recently, it was expanded to include patients with 1–3 positive lymph nodes. The patients are assessed by the standard clinicopathologic prognostic factors included in adjuvant! and by the 70-gene signature assay. If both traditional and molecular assays predict a high-risk status, then the patient will receive adjuvant cytotoxic chemotherapy (and hormonal therapy if the tumor is ER-positive). If both assays indicate a low risk, no chemotherapy is given and ER-positive patients are given adjuvant hormonal therapy only. When there is discordance between the traditional clinicopathologic prognostic factors and the 70-gene signature, patients are randomized to receive treatment based on either the genomic or the clinical predictive results. The results of this trial will provide more data about the use of the 70-gene signature in early-stage breast cancer.

5 The HOXB13:IL17BR Ratio

The anti-apoptotic homeobox B13 (HOXB13) gene and interleukin 17 B receptor (IL17BR) gene are used to calculate the HOXB13:IL17BR (H/I) expression ratio. This ratio was developed by a microarray-based screening of 22,000 genes in 60 patients with ER-positive, node-positive, or node-negative breast cancer, treated with tamoxifen (Ma et al. 2004). Three genes that were identified were significantly associated with clinical outcome: HOXB13, IL17BR, and CHDH (choline dehydrogenase). High expression of HOXB13 was associated with recurrence, while high expression of IL17BR and CHDH was associated with non-recurrence. A higher ratio of the HOXB13 and IL17BR strongly predicted recurrence, and pairing with CHDH did not provide additional predictive power. A larger validation study was done in 852 patients with stage I or II breast cancer with a median follow-up of 6.8 years (Ma et al. 2006). In this study, 286 (34 %) patients were tamoxifen-treated and 566 (66 %) patients were untreated. Of note, patients were not randomized to a treatment arm. The expression of HOXB13, IL17BR, and CHDH, as well as ER and PR were quantified by RT-PCR. Gene expression and clinical variables were analyzed for association with relapse-free survival. Expression of HOXB13 was associated with a shorter RFS ($p = 0.008$), whereas the expression of IL17BR and CHDH was associated with longer RFS ($p < 0.0001$ and $p = 0.0002$, respectively). In the ER-positive patients, the HOXB13:IL17BR index predicted clinical outcome, independently of treatment, but more strongly in the node-negative patients. This study also suggested a role for the H/I ratio as a prognostic test in untreated patients. The HOXB13/IL17BR ratio was tested but not verified in a retrospective

study of 58 ER-positive patients treated with tamoxifen for 5 years, most of whom were node-positive (77 %) (Reid et al. 2005). In this study, the H/I ratio failed to show a relationship between the expression of these genes and distant relapse or survival.

The association between the H/I ratio and clinical outcome was evaluated in tumor specimens from the NCCTG 89-30-52 trial (Goetz et al. 2006). In this trial, postmenopausal women with ER-positive breast cancer were randomized to 5 years of tamoxifen with or without 1 year of fluoxymesterone. For the 227 patients in the tamoxifen-only arm, RT-PCR profiles for HOXB13 and IL17BR were obtained from 206 paraffin-embedded tumor blocks. In the node-positive cohort ($n = 86$), the H/I ratio was not associated with relapse or survival. In contrast, in the node-negative cohort ($n = 130$), a high H/I ratio was associated with a significantly decreased relapse-free survival [HR, 1.98; $P = 0.031$], disease-free survival (HR, 2.03; $P = 0.015$), and overall survival (HR, 2.4; $P = 0.014$), independent of standard prognostic markers. One explanation could be that these genes may have a role in early invasion and metastatic potential, and therefore, they could be more relevant in the node-negative population. Similar findings regarding the nodal status were demonstrated in a large cohort ($N = 852$) of both untreated and tamoxifen-treated patients (Erlander et al. 2005). In this study, the H/I ratio was associated with relapse and death in node-negative but not node-positive, ER-positive patients.

In another study, the ability of the H/I ratio to predict disease-free survival was tested in 1,252 breast tumor specimens (Jansen et al. 2007). In 468 patients with node-negative, ER-positive breast cancer who did not receive adjuvant chemotherapy, the H/I ratio was significantly associated with poorer disease-free survival (HR, 1.6; $P = 0.02$) and poorer overall survival (HR not reported; $P < 0.001$). In a multivariate analysis of 151 untreated patients with ER-positive, node-positive breast cancer, an association was shown between the H/I ratio and overall survival ($p < 0.001$) but not disease-free survival ($p = 0.065$). In 193 patients treated with tamoxifen at first relapse, the ratio was significantly associated with progression-free survival. The authors concluded that higher H/I ratio expression levels are associated with both tumor aggressiveness and failure to respond to tamoxifen. One study investigated whether the H/I ratio predicted a difference in benefit between 264 patients with postmenopausal breast cancer who received tamoxifen for 2 and 5 years and 93 pre-menopausal patients who did not receive systemic therapy (Jerevall et al. 2008). In this study, 72 % of the patients had node-positive disease and 74 % had ER-positive disease. The HOXB13:IL17BR gene expression ratio and the expression of HOXB13 alone predicted the benefit of endocrine therapy, with a high ratio or a high expression

rendering patients less likely to respond. Neither the patient profile nor the methods of calculation of the ratio were identical to those used in previous studies. The results of this study differed from previous reports because, in this case, the H/I ratio was associated with outcomes in patients with lymph node-positive disease.

6 Theros Breast Cancer Index

Theros Breast Cancer Index (TBCI) is a prognostic profile that provides a quantitative assessment of the likelihood of distant recurrence in patients with ER-positive, node-negative breast cancer. It is a molecular assay that combines two indices—HOXB13:IL17BR and a five-gene molecular grade index (MGI). The MGI is a gene expression index for tumor grade that includes 5 cell cycle-related genes. It was generated using two microarray data sets testing a total of 410 patients (Ma et al. 2008). A 323-patient cohort was used to develop an RT-PCR assay for MGI and to validate its prognostic utility. When combined with the HOXB13: IL17BR index, it was noted that the two assays modified the prognostic performance of each other. A high MGI was associated with a significantly worse outcome only in combination with a high HOXB13:IL17BR, and likewise, a high HOXB13:IL17BR was significantly associated with a poor outcome only in combination with a high MGI.

The TBCI was further assessed in a retrospective study of 262 patients with ER-positive, node-negative breast cancer with at least a 10-year follow-up (Jankowitz et al. 2010). The TBCI was compared to adjuvant! online to see whether it added additional predictive power for recurrence and overall survival. The TBCI predicted breast cancer recurrence and overall survival more accurately than adjuvant! online combined with and traditional clinical and pathologic features. Both TBCI and adjuvant! online retained independent prognostic significance for recurrence and death in a multivariate analysis, indicating that the two tests can provide complementary information.

7 Rotterdam 76-Gene Signature

A 76-gene signature was developed using 286 tumor samples of node-negative breast cancers from a single institution (Wang et al. 2005). In a training set of 115 tumors, gene expression analysis led to the identification of a 76-gene signature consisting of 60 genes for ER-positive tumors and 16 genes for ER-negative tumors. The 76-gene signature showed 93 % sensitivity and 48 % specificity in a subsequent independent testing set of 171 lymph node-negative patients. The gene profile identified patients who developed distant metastases within 5 years (HR 5.67 [95 % CI

2.59–12.4]), even when corrected for the traditional prognostic factors in a multivariate analysis (5.55 [2.46–12.5]). After 5 years, the absolute differences in distant metastasis-free and overall survival between the patients with the good or poor 76-gene signatures were 40 and 27 %, respectively. Among the patients with good-prognosis signatures, 7 % developed distant metastases and 3 % died within 5 years. The 76-gene profile also provided significant prognostic information regarding the development of metastasis in premenopausal patients (84 patients), postmenopausal patients (87 patients), and patients with tumors measuring 10–20 mm. In this series, the assay also outperformed the St. Gallen's (Goldhirsch et al. 2001, 2005) and NIH guidelines (NIH Consensus Statement Online 2000; Eifel et al. 2001) for the identification of patients with a good prognosis.

The 76-gene signature profile was further validated in an independent multicenter study of 180 patients with node-negative breast cancer who did not receive adjuvant systemic therapy (Foekens et al. 2006). In this study, frozen samples were analyzed by quantitative RT-PCR rather than microarray analysis. The 76-gene signature was highly accurate in identifying patients who would develop distant metastasis within 5 years (HR, 7.41; 95 % CI, 2.63–20.9), even when corrected for traditional prognostic factors in a multivariate analysis (HR, 11.36; 95 % CI, 2.67–48.4). The actuarial 5-and 10-year distant metastasis-free survival rates were 96 % (95 % CI, 89–99 %) and 94 % (95 % CI, 83–98 %), respectively, for the good-profile group and 74 % (95 % CI, 64 % to 81 %) and 65 % (53–74 %), respectively for the poor-profile group. The 76-gene signature was confirmed as a strong prognostic factor in subgroups of ER-positive patients, premenopausal and postmenopausal patients, and patients with tumors that were 20 mm or smaller. The subgroup of patients with ER-negative tumors was too small to perform a separate analysis.

Like the 70-gene signature, this assay requires fresh or frozen tissue, and the prognostic information relies primarily on the degree of expression of proliferation-related genes. It may not be useful in assessing the outcome in patients with ER-negative, HER2-negative cancers (Wirapati et al. 2008; Desmedt et al. 2007).

8 Mammostrat

Mammostrat® is an immunohistochemical (IHC) assay that measures five biomarkers: SLC7A5, HTF9C, P53, NDRG1, and CEACAM5. This test could potentially be implemented in the routine pathologic assessment of breast cancers because it is performed using IHC. The biomarkers are independent of one another and do not directly measure either proliferation or hormone receptor status. They were selected from gene expression data which guided the

production of hundreds of novel antibody reagents (Ring et al. 2006). Five reagents (p53, NDRG1, CEACAM5, SLC7A5, and HTF9C) were shown to identify ER-positive patients with poor outcomes. The assay was then tested in a blinded, retrospective study using tissue arrays of paraffin blocks from the ER-positive, node-negative samples from the NSABP B14 and B20 trials (Ross et al. 2008). Tissue arrays were stained by IHC, targeting the 5 biomarkers, and risk stratification was done using predefined scoring rules, an algorithm for combining scores, and cutoff points for low-risk, moderate-risk, and high-risk patient groups. In a multivariate model, the IHC assay contributed information independent of age, tumor size, or menopausal status ($P = 0.007$). The Kaplan–Meier estimates for recurrence-free survival after 10 years were 73, 86, and 85 % for the high-risk, moderate-risk, and low-risk groups ($P = 0.001$), and the breast cancer-specific death rates were 23, 10, and 9 % ($P < 0.0001$), respectively. Both high-risk and low-risk groups showed significant improvement with cytotoxic chemotherapy. However, the magnitude of benefit in the high-risk patients was four times greater than in the low-risk patients. The largest validation of this assay was done in a single institution series from 1981 to 1998. 1,812 women with early-stage breast cancer were studied, and 1,390 cases were assayed (Bartlett et al. 2010). Each case was assigned a Mammostrat® risk score, and distant recurrence-free survival (DRFS), RFS, and overall survival (OS) were analyzed by marker positivity and risk score. An increased Mammostrat® score was significantly associated with reduced DRFS, RFS, and OS in patients with ER-positive breast cancer ($P < 0.00001$). In node-negative, tamoxifen-treated patients, 10-year recurrence rates were 7.6 ± 1.5 % in the low-risk group vs. 20.0 ± 4.4 % in the high-risk group.

9   PAM-50

PAM-50 is a 50-gene assay using quantitative RT-PCR, developed to identify intrinsic breast cancer subtypes (luminal A/B, HER2-enriched, basal-like). It also includes genes related to proliferation and tumor size. It can be performed on archival breast tissue. A risk of relapse (ROR) score is generated for all patients, including those with ER-negative disease. In a test set of 761 patients who did not receive any systemic therapy, the intrinsic subtypes showed prognostic significance ($P = 2.26E-12$) and the results remained significant in multivariable analyses that incorporated standard parameters, including ER status, histological grade, tumor size, and node status (Parker et al. 2009). A combined model of subtype and tumor size was a significant improvement on either the clinical/pathological model or subtype model alone. In a set of 133 patients treated with neoadjuvant anthracycline and taxanes, the intrinsic subtype model predicted neoadjuvant chemotherapy efficacy with a negative predictive value for pathologic complete response of 97 %.

In a series of 786 patients with ER-positive breast cancer, treated with tamoxifen, the PAM50 qRT-PCR signatures performed on formalin-fixed, paraffin-embedded tissue gave more prognostic information than clinical assays for hormone receptors or Ki-67 (Nielsen et al. 2010). In node-negative patients, PAM50 qRT-PCR-based risk assignment weighted for tumor size and proliferation identified a group with >95 % 10-year survival without chemotherapy. In node-positive patients, PAM50-based prognostic models were also superior.

The PAM50 risk of recurrence (ROR) score was evaluated in the TransATAC population (ER-positive, node-negative, and node-positive women treated with anastrozole or tamoxifen), and compared with the OncotypeDx and a composite IHC score (IHC4), including ER, PR, HER2, and Ki-67 (Dowsett et al. 2011). In this study, PAM-50 provided greater prognostic information than the OncotypeDx RS, and as much information as the IHC4. PAM50 was prognostic for 10-year distant recurrence in the overall population, and in node-positive, node-negative, and HER2-negative patients. Similar results were seen with a 46-gene variation (PAM-46).

10   EndoPredict—11 Genes

The EndoPredict (EP) assay evaluates eight cancer-related and three reference genes using quantitative RT-PCR on formalin-fixed, paraffin-embedded tissue (Filipits et al. 2011). A risk score is calculated, and the score is either low or high. The EP score was combined with nodal status and tumor size into a comprehensive risk score–EPclin. The test is designed to assess the risk of distant recurrence in patients with ER-positive, HER2-negative breast cancer treated with endocrine therapy alone. The test was validated using samples from two trials: the Austrian Breast and Colorectal Cancer Study Group (ABCSG)-6 ($n = 378$, tamoxifen-only-treated patients) and ABCSG-8 ($n = 1,324$, patients treated with either tamoxifen for 5 years or tamoxifen for 2 years followed by anastrozole for 3 additional years). In both of these cohorts, the continuous EP was an independent predictor of distant recurrence in multivariate analysis (ABCSG-6 $p = 0.010$, ABCSG-8 $p < 0.001$). The test provided prognostic information independent of, and in addition to, clinicopathologic variables, in particular, adjuvant! online and the Ki-67 labeling index.

11   Genomic Grade Index–97 Genes

The genomic grade index (GGI) uses a 97-gene assay to measure the histological tumor grade. The test is based on the premise that the histological grade is a strong prognostic factor in ER-positive tumors, and that the reproducibility of histological grade is suboptimal. The GGI is capable of dividing breast cancers of intermediate grade into two groups, grade I-like, which have a low frequency of distant relapses, and grade III-like, which have a clinical behavior similar to grade III cancers (Sotiriou et al. 2006). Similar results were obtained in an analysis of 347 tumor samples, where it was found that the genomic grade was an independent prognostic indicator of disease recurrence (Ivshina et al. 2006). In another study, 666 ER-positive tumors were classified into high and low genomic grade using the GGI (Loi et al. 2007). These were highly comparable to the previously described luminal A and B classification and significantly correlated with the risk groups generated using the 21-gene RS. The two subtypes were associated with statistically distinct clinical outcome in both tamoxifen-treated and tamoxifen-untreated populations.

The value of the GGI in predicting the response to neo-adjuvant chemotherapy in patients with HER-2–normal breast cancer was reported by investigators from the MD Anderson Cancer Center (Liedtke et al. 2009). Gene expression data were generated from fine-needle aspiration biopsies performed on 229 patients prospectively collected before neoadjuvant paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide chemotherapy. Eighty-five percent of grade 1 tumors had low GGI, 89 % of grade 3 tumors had high GGI, and 63 % of grade 2 tumors had low GGI. In both ER-negative and ER-positive patients, a high GGI was associated with a pathologic complete response or minimal residual disease, demonstrating an increased sensitivity to chemotherapy. High GGI was also associated with a significantly worse distant RFS in patients with ER-positive cancer ($p = 0.005$).

Initially, GGI required fresh or frozen samples. However, a modified version of this signature based on qRT-PCR analysis has recently been developed (Toussaint et al. 2009). The prognostic information provided by GGI is applicable only to ER-positive breast cancer (Wirapati et al. 2008; Desmedt et al. 2007).

12   Wound-Response Gene Expression Signature

Core serum response genes are a set of genes closely associated with wound healing and cancer progression. The gene expression profile of fibroblasts activated in the serum is also expressed in tumors by the tumor cells, by tumor-associated fibroblasts, or both. The expression of the wound-response signature was shown to be associated with poor overall survival and an increased risk of metastasis in common epithelial tumors, such as breast, lung, and gastric cancers (Chang et al. 2004). Measurements of genes in this profile show a biphasic pattern of gene expression, with either an activated or quiescent wound-response signature. This profile was validated in the same group of 295 early breast cancer patients that was used to validate the 70-gene signature (Chang et al. 2005). A univariate analysis of the patients showed that the activated signature was associated with a decreased distant metastasis-free and overall survival. The wound-response signature gave more accurate risk stratification independently of known clinical and pathologic risk factors. The 70-gene prognosis signature and intrinsic molecular subtype classification (Perou et al. 2000) were also performed on these tumor samples, and the results from the different gene signatures were overlapping and were all consistent predictors of outcome. Prospective studies are needed to determine whether treatment decisions based on the wound-response signature will benefit patients clinically.

13   Comparison of Different Genetic Profiles

As discussed in this chapter, many studies of gene expression in breast cancer have identified expression profiles and gene sets that are prognostic, predictive, or both. The genes evaluated in these different profiles show only minimal overlap. The reasons for this are not completely understood, but probably include differences in patient cohorts, microarray platforms, and methods of statistical analysis.

There is little data about head-to-head comparisons of the different molecular profiles. However, five profiles were compared in one data set of 295 samples, where information was available about RFS and overall survival (OS). These profiles were the 70-gene signature, the wound-response gene set, the 21-gene RS, the intrinsic subtype model, and the HOXB13/IL17BR two-gene ratio (Fan et al. 2006). These were the same 295 samples that had been used to develop the 70-gene signature (van de Vijver et al. 2002). The RS and two-gene ratio were described as a derived score and estimated from microarray gene expression data rather than qRT-PCR. Therefore, they were not obtained according to the protocols and methods used in the marketed assays. Each of the five gene expression profiles, except for the two-gene ratio, was a significant predictor of RFS and OS, as were ER status, tumor grade, tumor diameter, and stage. This was also true for the 225 tumors that were ER-positive. Each profile, except the two-gene ratio, added important prognostic information beyond the standard clinical predictors.

Table 2 Summary of available prognostic and predictive gene expression signatures

Test	Type of assay	Type of tissue sample	Indication	FDA clearance	ASCO recommended
Oncotype DX	21-gene recurrence score	Formalin-fixed paraffin-embedded	Prognosis and prediction of benefit from chemotherapy in ER+ N0/1–3N+ breast cancer on tamoxifen use	No	Yes
MammaPrint	70-gene signature	Fresh frozen tumor samples	Prognosis in N0, <5 cm tumor diameter, stage I/II, ER± breast cancer	Yes	No
H/I ratio	2-gene expression ratio	Formalin-fixed paraffin-embedded	ER+, N±, stage I/II breast cancer on tamoxifen use	No	No
Theros Breast Cancer Index	Combination of H/I ratio and 5-gene molecular grade index into a breast cancer index	Formalin-fixed paraffin-embedded	Prognosis and prediction of response to tamoxifen in ER+ breast cancer	No	No
EndoPredict	11-gene score	Formalin-fixed paraffin-embedded	ER+, HER2− breast cancer treated with endocrine therapy	No	No
Genomic grade index	97-gene genomic grade index	Frozen or formalin-fixed paraffin-embedded	ER+, histological grade II breast cancer	No	No
Rotterdam 76-gene signature	76-gene signature	Fresh frozen tumor samples	ER±, N0 breast cancer	No	No
Mammostrat	Immunohistochemical assay measuring five biomarkers	Formalin-fixed paraffin-embedded	ER+, N0 breast cancer	No	No
PAM-50	50-gene signature	Formalin-fixed paraffin-embedded	Prognosis in ER+, N ± breast cancer	No	No

Estrogen receptor (ER); Lymph node (N)

Each profile was analyzed relative to the intrinsic subtype classification. All 53 tumors with basal-like subtype were found to have a poor 70-gene signature profile and a high RS, and 50 tumors had an activated wound-response signature. This was also true for the tumors with the HER2-positive, ER-negative subtypes, as well as for the poor-outcome luminal B subtype that is classified as ER positive. Conversely, the normal-like and luminal A tumors showed heterogeneity in terms of how they were classified by the other models. However, 62 of 70 samples with low RS were of the luminal A subtype. These results suggest that if a sample is classified as basal-like, HER2-positive, and ER-negative, or luminal B, then it would most likely be in the poor-prognosis groups of the 70-gene, wound-response, and recurrence score models. Pairwise comparisons of the 70-gene, wound-response, recurrence score, and two-gene models showed that the results of all but the two-gene model were highly concordant. Since the 70-gene signature and the RS model are the best validated, they were directly compared, with low and intermediate RSs considered equivalent to a good 70-gene signature and a high RS to be equivalent to a poor 70-gene signature. In the entire group of patients,

there was 81 % agreement (239/295 patients), and in the ER-positive subset, there was a 77 % agreement (173/225 patients). These results suggest that even though there is very little overlap in the genes that are analyzed (the 70-gene and the RS profiles overlapped by only 1 gene: *SCUBE2*) and different algorithms are used, the outcome predictions provided by these profiles are similar for the majority of patients. The profiles probably reflect common cellular phenotypes and biological characteristics in the different groups of breast cancer patients.

In a comprehensive meta-analysis integrating both clinical, pathological, and gene expression data in over 2,100 patients, a multivariate analysis showed that in the ER+/HER2− subgroup, only the proliferation module and the histological grade were significantly associated with clinical outcome (Desmedt et al. 2008). In the ER−/HER2− subgroup, only the immune response module was associated with prognosis, whereas in the HER2+ tumors, the tumor invasion and immune response modules displayed a significant association with survival. Proliferation was identified as the most important component of the prognostic signatures, and the performance was limited to the ER+/HER2-

subgroup. In another meta-analysis of 2,833 breast cancers, gene coexpression modules of three key biological processes (proliferation, ER signaling and HER2 signaling) were used to analyze the genes of nine prognostic signatures (Wirapati et al. 2008). All nine prognostic signatures had a similar prognostic performance in the entire data set, mostly due to the detection of proliferation activity. In this study, ER status and ERBB2 expression seemed to correspond with a poor outcome, due to elevated expression of proliferation genes. Also, clinical variables, such as tumor size and nodal status, added additional independent prognostic information (Table 2).

14 Limitations

The gene expression prognostic signatures share several characteristics (Reis-Filho and Pusztai 2011). Despite the differences in the genes they measure, the gene signature tests identify the same group of patients as having a poor prognosis. The unifying characteristic is the high expression of proliferation-related genes (Wirapati et al. 2008). When gene signatures were divided into two subsignatures (Wirapati et al. 2008; Reyal et al. 2008) one composed of only the proliferation-related genes and the other composed of the remaining genes, the proliferation-related subsignatures had a prognostic impact as strong or stronger than the original signatures (Wirapati et al. 2008; Reyal et al. 2008). Importantly, the subsignatures composed of non-proliferation-related genes were shown not to have a prognostic impact (Wirapati et al. 2008). This is especially true in ER-positive breast cancers, where the level of expression of proliferation-related genes is one of the strongest prognostic factors. In ER-negative cancers, the expression of proliferation-related genes is usually high and, therefore, gene signatures have failed to stratify ER-negative breast cancers into separate prognostic subgroups (Mook et al. 2009, 2010; Wirapati et al. 2008). Even adenoid cystic carcinoma of the breast, which is ER-negative and has an indolent clinical course, is classified by gene signatures as having a poor prognosis (Weigelt et al. 2008). Chemotherapy targets rapidly proliferating cells, and since the gene signatures predominantly measure proliferation-related genes, most of the gene signature assays also correlate with response to chemotherapy. In addition, meta-analyses have shown that tumor size and lymph node status provide prognostic information that is independent of the results from the prognostic signatures. The accuracy of the predictions of the prognostic signatures seems to be time-dependent, with more accurate predictions seen at 5 years than at 10 years after diagnosis.

The genetic profiles use a variety of techniques to perform the assays in the reported studies. All studies of 21-gene RT-PCR assay have used the commercial test as opposed to the signature, whereas the studies of the 70-gene signature have used either the signature or the assay. Only the large multicenter validation by (Buyse et al. 2006) used the marketed MammaPrint assay. The study that compared the results of the marketed MammaPrint test to the signature on the same samples showed that about 9 % of the patients were placed into different risk groups when the marketed test was used (Glas et al. 2006). Almost all of the studies that looked at the H/I ratio calculated the test in slightly different ways.

The accurate use of these tests in clinical decision making will be vitally important. Only the 21-gene RS has been shown to be prognostic as well as predictive for a benefit from both tamoxifen and chemotherapy. This was based on data from the randomized, clinical trials, NSABP B-14 and B-20 (Paik et al. 2004, 2006). The other genomic tests are purely prognostic and indicate the likely outcome, independent of therapy. These tests have limited data regarding their predictive abilities, but it is assumed that patients who have a low risk of recurrence or death may forgo chemotherapy.

The study populations that were used to validate the various gene signature tests were not uniform. The 21-gene RS used the most clinically and therapeutically homogeneous population. This is reflected in the inclusion criteria for the test: patients with ER-positive, lymph node-negative, stage I or II disease who receive tamoxifen. The 70-gene signature was tested in a more heterogeneous population, with both lymph node-positive and lymph node-negative patients, and patients with ER-positive and ER-negative diseases.

15 Conclusion

In early-stage breast cancer, the standard approach has been to use clinical and pathological variables, such as tumor size, lymph node metastases, tumor grade, ER, PR, and HER2 status to create treatment guidelines (e.g., adjuvant! online, the NCCN guidelines, the NIH Consensus Development Criteria, and the St. Gallen criteria). These guidelines provide risk classifications which help guide decisions regarding adjuvant therapy. These approaches have been successful, and there has been a steady reduction in breast cancer mortality over the past three decades. However, with the development of individualized medicine, genomic signatures can provide a more accurate assessment of the risk of recurrence and the benefit of adjuvant therapy.

Recently, many gene expression profiles have been developed for breast cancer. Several of these tests are now commercially available, and more new tests are being developed. These gene expression profiles appear to provide additional prognostic and predictive information that complements the traditional clinical and pathological parameters. However, most of the genetic profiles have not been validated in prospective randomized trials. Also, there have not been any large, prospective, head-to-head comparisons between the different tests. Each test looks at a different set of genes, and there is little overlap in the tested genes. Despite the lack of overlap, these tests identify the same group of patients as having a poor prognosis, probably because all of the signatures rely heavily on proliferation-related genes (Wirapati et al. 2008). The tests are often only informative in ER-positive breast cancers, which limits their utility. Also, the tests are expensive and may have limited availability.

With so many tests available or in development, some important questions need to be answered: Is the test accurate and reliable? Does the evidence show that it is strongly prognostic? Is there evidence that the test result is predictive of chemotherapy benefit? What is the level of evidence for the accuracy of the assay? Has the assay been incorporated into treatment guidelines?

In spite of all these caveats, the new genetic signatures almost uniformly allow a more accurate prediction of outcomes in breast cancer patients. As the tests are improved, and comparison studies are conducted, the role of the various profiles and the importance of individual genes in the profiles will be better understood. The results from these tests will allow oncologists to spare many women from the toxicities and long-term side effects of unnecessary adjuvant chemotherapy. These multigene assays will contribute to major improvements in the treatment of breast cancer.

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