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New Molecular Classifications of Breast Cancer

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Abstract

Traditionally, pathologic determinations of tumor size, lymph node status, endocrine receptor status, and human epidermal growth factor receptor 2 (HER2) status have driven prognostic predictions and adjuvant therapy recommendations for patients with early stage breast cancer. However, these prognostic and predictive factors are relatively crude measures, resulting in many patients being overtreated or undertreated. As a result of gene expression assays, there is growing recognition that breast cancer is a molecularly heterogeneous disease. Evidence from gene expression microarrays suggests the presence of multiple molecular subtypes of breast cancer. The recent commercial availability of gene expression profiling techniques that predict risk of disease recurrence as well as potential chemotherapy benefit have shown promise in refining clinical decision making. These techniques will be reviewed in this article. CA Cancer J Clin 2009;59:303–313. ©2009 American Cancer Society, Inc.

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Introduction

Clinically, breast cancer is a remarkably heterogeneous disease. Traditionally, pathologic determinations of tumor size, lymph node status, endocrine receptor status, and human epidermal growth factor receptor 2 (HER2) status have driven prognostic predictions and, ultimately, adjuvant therapy recommendations for patients with early stage breast cancer. However, these prognostic and predictive factors are relatively crude measures and many patients are overtreated or undertreated as a result. Using data from the Surveillance, Epidemiology, and End Results (SEER) database and the results of individual clinical trials, Ravdin et al. developed a widely used, computerized model called Adjuvant! Online, an online source (available at: http://www.adjuvantonline.com, Accessed July 27, 2009) to facilitate clinical decision making.[1] In an independent validation using data from the British Columbia Breast Cancer Outcomes Unit, Adjuvant! Online performed reliably. However, although predicted and observed outcomes were within 2% for the majority of the demographic, pathologic, and treatment-defined subgroups, Adjuvant! Online overestimated overall survival (OS), breast cancer-specific survival, and event-free survival for women aged younger than 35 years and for patients with tumors with lymphovascular or vascular invasion.[2] Recently, gene expression profiling techniques that predict risk of disease recurrence as well as potential chemotherapy benefit have shown promise in refining clinical decision making.

Breast Cancer Molecular Subtypes

Evidence from gene expression microarrays suggests the presence of multiple molecular subtypes of breast cancer. Using complementary DNA (cDNA) microarrays representing 8,102 human genes to characterize gene expres-

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sion patterns in a set of 65 surgical specimens of human breast tumors from 42 different individuals, Perou et al demonstrated that the phenotypic diversity of breast tumors was associated with corresponding gene expression diversity.[3] From the genes in the 65 tissues samples, the investigators selected a subset of 456 genes, which were termed the "intrinsic" gene subset, and consisted of genes with significantly greater expression variation between different tumors than between paired samples from the same tumor. Using this subset, the authors were then able to identify 4 different molecular subtypes of breast cancer: estrogen receptor (ER)-positive/luminal-like, basal-like, Erb–B2-positive, and normal breast. Subsequent data expanded the classification to distinguish between luminal A and luminal B.[4] These 5 molecular subtypes have been confirmed in independent data sets[5] and, importantly, the gene expression subtype appears consistent between primary tumors and subsequent metastatic lesions occurring years later.[6] Furthermore, the subtypes are associated with differences in clinical outcome. Sorlie et al examined a subset of 49 patients with locally advanced breast cancer who were treated with doxorubicin and had a median follow-up of 66 months and found that the recurrence-free survival (RFS) and OS differed significantly among the breast cancer subtypes, with the luminal A tumors having the longest survival times, the basal-like and HER2-positive subtypes having the shortest survival times, and the luminal B tumors having an intermediate survival time.[4]

Recently, a risk model incorporating the gene expression-based luminal A and B, HER2-positive, and basal-like subtypes was developed by Parker et al.[7] Using microarray and quantitative reverse transcriptase-polymerase chain reaction (RT–PCR) data from 189 samples, a 50-gene subtype predictor was developed and evaluated in 2 cohorts of patients: a cohort of patients receiving no adjuvant systemic therapy and a cohort of patients undergoing neoadjuvant chemotherapy with paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide. Test sets from 761 patients who did not receive systemic therapy were evaluated for prognosis and 133 samples from patients who received neoadjuvant chemotherapy were evaluated for prediction of a pathologic complete response (pCR) after neodjuvant chemotherapy.

Among the 626 ER-positive tumors studied, 73% were luminal A or B, 11% were HER2-enriched, 5% were basal-like, and 12% were normal breast. In contrast, among the ER-negative tumors, 11% were luminal A or B, 32% were HER2-enriched, 50% were basal-like, and 7% were normal breast. The intrinsic subtypes as distinct entities were found to have a significant impact on RFS in the untreated patients and remained significant in multivariate analysis incorporating standard prognostic factors such as ER status, histologic grade, tumor size, and lymph node status. Furthermore, the intrinsic subtype model predicted the likelihood of a pCR after neoadjuvant chemotherapy, with a sensitivity and specificity of 94% and 57%, respectively. The positive and negative predictive values were 43.2% and 96.6%, respectively. However, there were significant discrepancies between the clinical classification of the tumors and the classification based on intrinsic subtypes. For example, of the 626 ER-positive tumors analyzed in the microarray test set, 5% were found to be basal-like. Of the 33 HER2-positive tumors, only 64% were classified as HER2-enriched by gene expression and 6% were classified as basal-like. Furthermore, 9% of the HER2-negative tumors were classified as HER2-enriched by gene expression. The authors' conclusions were that ER and HER2 status are not accurate surrogates for the true intrinsic subtype status.[7] However, this raises important questions with regard to the optimal classification system to guide therapeutic decision making.

Gene Expression Profiling Assays

The 70-Gene Assay (MammaPrint)

Using inkjet-synthesized oligonucleotide microarrays on primary breast tumors from 117 patients aged younger than 55 years, investigators from the Netherlands Cancer Institute identified a gene expression profile based on 70 genes associated with prognosis in patients with lymph node-negative breast cancer.[8] The odds ratio for the development of metastatic disease from a tumor with a poor-prognosis gene signature compared with a tumor with a good-prognosis gene signature was approximately 15. To validate the profile, a cohort of 295 consecutive patients aged younger than 53 years with stage I or II breast cancer (151 with lymph node-negative disease and

144 with lymph node-positive disease) were evaluated and classified as having either a poor or good prognosis profile.[9] There were 69 ER-negative tumors and 226 ER-positive tumors. Among the 295 patients, 180 were classified as having a poor-prognosis signature and 115 as having a good-prognosis signature, with mean (+/− SE) overall 10-year survival rates of 54.6% ±4.4% and 94.5% ±2.6%, respectively. At 10 years, the probability of remaining free of distant metastasis was 50.6% ±4.5% for the group with a poor-prognosis signature and 85.2% ±4.3% for the group with a good-prognosis signature. The hazard ratio (HR) for distant metastases in the poor-prognosis group compared with the good-prognosis group was 5.1 (95% confidence interval [95% CI], 2.9−9.0; $P < .001$) and this ratio remained significant when analyzed according to lymph node status. Furthermore, on multivariate analysis, the prognosis profile was found to be a strong independent predictor of the likelihood of distant metastases (HR, 4.6; 95% CI, 2.3–9.2 [$P < .001$]).

The assay was further validated by the Translational Breast International Group (TRANSBIG) research consortium in a retrospective study of frozen, archival tumor material collected from 302 patients with lymph node-negative disease from 5 non-Dutch cancer centers.[10] All the patients were aged 60 years or younger and had lymph node-negative, T1 or T2 tumors, and the majority of patients had not received systemic adjuvant therapy. The median follow-up was 13.6 years. The 70-gene prognosis profile was found to be a significant prognostic indicator of both distant disease-free survival (DDFS) and OS in this group of patients.

There are emerging data addressing the ability of the 70-gene assay to predict chemotherapy benefit. Recently, a pooled analysis of 1,637 patients collected from 7 large data sets at multiple institutions across Europe was reported.[11] In this meta-analysis, the 70-gene assay assigned 772 patients (47%) to the "low-risk" category and 865 patients (53%) to the "high-risk" category. Among these patients, 349 were treated with endocrine therapy alone, whereas 226 were treated with both chemotherapy and endocrine therapy. Patients with a poor-prognosis 70-gene profile appeared to derive a significant benefit from the addition of chemotherapy. DDFS was improved from 69% to 88% (HR, 0.28; 95% CI, 0.14−0.56; [$P < .001$]). Conversely, patients with a good-prognosis

70-gene profile did not appear to derive a significant benefit from chemotherapy ($P = .962$). However, a limitation of this analysis was the relatively small number of events in the "low-risk" group of patients.

The 70-gene assay requires fresh mRNA for analysis (fresh-frozen tumor samples or tissues collected in an RNA preservative solution).

76-Gene Assay

Investigators from Rotterdam, the Netherlands, identified a 76-gene signature (60 genes for patients with ER-positive disease and 16 genes for patients with ER-negative disease) in a training set of 115 tumors.[12] In an independent testing set of 171 patients with lymph node-negative disease, this signature demonstrated 93% sensitivity and 48% specificity in identifying patients who developed distant metastatic disease within 5 years (HR, 5.67; 95% CI, 2.59−12.4). At 80 months, the absolute difference between the patients with a good and those with a poor prognosis was 39% (88% vs 49%) for DDFS and 27% (97% vs 70%) for OS. Subgroup analysis demonstrated the profile to be a strong prognostic factor for both premenopausal and postmenopausal women, as well as women with small tumors (those measuring 1–2 cm).

This signature was subsequently validated in an independent, multi-institutional set of tumor samples from 180 patients with lymph node-negative disease who did not receive adjuvant systemic therapy.[13] In this group, the 5-year and 10-year DDFS rates were 96% and 94%, respectively, for the good-profile group and 74% and 65%, respectively, for the poor-profile group. The sensitivity and specificity for 5-year DDFS were 90% and 50%, respectively. This analysis confirmed the signature to be a strong prognostic factor in the subgroups of ER-positive patients and both premenopausal and postmenopausal patients, as well as those with a tumor size ≤20 mm. However, the subgroup of patients with ER-negative tumors was too small for analysis.

The 76-gene assay also requires fresh or frozen extracted mRNA, similar to the 70-gene assay.

The HOXB13:IL17BR Assay

Ma et al performed microarray gene expression analysis of 60 tumors identified from a total of 103 patients with ER-positive, early stage breast cancer who presented to Massachusetts General Hospital

TABLE 1. Validation Studies for the HOXB13:IL17BR Assay

TRIAL	POPULATION	RESULTS
North Central Cancer Treatment Group (NCCTG) 89-30-52 (Goetz 2006)[16]	211 postmenopausal women with ER-positive, early stage breast cancer who were treated with tamoxifen.	Lymph node-positive patients: no association between the HOXB13:IL17BR expression ratio and RFS or OS Lymph node-negative patients: a high HOXB13:IL17BR expression ratio was associated with worse RFS and OS compared with a low ratio.
Tumor Bank and Data Network Core at the Breast Center of Baylor College of Medicine (Ma 2006)[17]	852 patients with stage I or stage II breast cancer who were treated with tamoxifen and 286 patients with stage I or stage II breast cancer who did not receive tamoxifen who were diagnosed between 1973 and 1993.	The HOXB13:IL17BR expression ratio predicted clinical outcome independently of tamoxifen treatment in the patients with ER-positive disease. Its prognostic ability was stronger in patients with lymph node-negative disease. In the subgroup of patients with ER-positive, lymph node-negative disease, multivariate analysis demonstrated the expression ratio to be a significant predictor of RFS (HR, 3.9; 95% CI, 1.5-10.3 [P = .007]).
Rotterdam cohort (Jansen 2007)[18]	1,252 patients with ER-positive, operable breast cancer. A total of 468 patients with ER-positive, primary breast cancer were analyzed, 217 (46%) of whom developed disease recurrence during the follow-up period. Expression levels were also evaluated in 193 patients with ER-positive, primary breast cancer who developed disease recurrence and were treated with first-line tamoxifen therapy.	The HOXB13:IL17BR expression ratio was found to be significantly associated with poor DFS and OS. The expression ratio was found to be significantly associated with poor response to tamoxifen (P = .027) and short PFS (P < .001).

ER indicates estrogen receptor; RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval; DFS, disease-free survival; PFS, progression-free survival.

between 1987 and 1997.[14] All the women were treated with adjuvant tamoxifen alone. A 2-gene expression ratio comprised of the homeobox gene HOXB13 and the interleukin–17B receptor IL17BR (HOXB13:IL17BR) was generated and found to be predictive of disease-free survival (DFS). HOX genes control morphogenesis and also play a role in maintaining tissue specificity.[15] HOXB13 may interact with the ER receptor and therefore overexpression may contribute to tamoxifen resistance. The role of IL17BR in breast cancer is less clear. The IL17BR gene, located at 3p21, is frequently lost in breast cancer. It has been hypothesized that one explanation for the correlation between IL17BR and prognosis is that low expression of the gene correlates with loss of tumor suppressor genes at 3p21.

The HOXB13:IL17BR ratio was validated in several population data sets (Table 1).[16–18] It was initially validated using the North Central Cancer Treatment Group (NCCTG) 89–30–52 trial, an adjuvant tamoxifen trial.[16] The NCCTG 89–30–52 trial randomized 541 postmenopausal women with ER-positive, early stage breast cancer to receive tamoxifen for 5 years or tamoxifen for 5 years plus fluoxymesterone for 1 year. Tumor blocks were obtained from 211 of the 256 patients treated with tamoxifen alone and RT–PCR profiles for HOXB13 and IL17BR were obtained from 206 patients. The HOXB13:IL17BR expression ratio was not found to be associated with relapse or survival in the lymph node-positive (n = 86) group of patients. However, in the lymph node-negative group (n = 130), a high ratio was associated with a significantly worse RFS (HR, 1.98; P = .031), DFS (HR, 2.03; P = .015), and OS (HR, 2.4; P = .014) compared with a low HOXB13:IL17BR expression ratio.

The HOXB13:IL17BR expression ratio was subsequently validated in a larger, independent patient cohort using data from the Tumor Bank and Data Network Core at the Breast Center of the Baylor College of Medicine in Houston, Texas.[17] Expression of HOXB13 and IL17BR were quantified using RT–PCR in 852 patients not treated with tamoxifen and 286 patients who did receive tamoxifen treatment. The HOXB13:IL17BR expression ratio was found to be predictive of clinical outcome independent of tamoxifen treatment in the group of patient with ER-positive disease; however, its prognostic

TABLE 2. Validation Studies for Prognostic Value of the 21-Gene Recurrence Score Assay

VALIDATION STUDY	POPULATION	RISK CATEGORY RESULTS	RATE OF DISTANT DISEASE RECURRENCE AT 10 YEARS	RISK OF BREAST CANCER-RELATED DEATH AT 10 YEARS
NSABP B–14 (Paik 2005)[24]	2,892 women with ER-positive, lymph node-negative breast cancer were randomized to 5 y of tamoxifen or placebo; an additional 1,235 women were assigned to receive an additional 5 y of tamoxifen. RT-PCR was performed on 668 samples.	Tamoxifen-treated patients: Low risk: 51% (RS <18), Intermediate risk: 22% (RS, 18-31), High risk: 27% (RS >31).	Low risk: 6.8% Intermediate risk: 14.3% High risk: 30.5%.	
Kaiser Permanente Study (Habel 2006)[25]	Case-control study of 4,964 women not treated with adjuvant chemotherapy. 220 patients who died of breast cancer and 570 matched controls.	Among 55 cases, 150 controls, tamoxifen-treated patients: Low risk: 29% (RS <18). Intermediate risk: 40% (RS, 18-31). High risk: 31% (RS >31).		ER-positive patients treated with tamoxifen: Low risk: 2.8% Intermediate risk: 10.7% High risk: 15.5%
The University of Texas M. D. Anderson Cancer Center Study (Esteva 2005)[26]	149 patients with lymph node-negative breast cancer who had not received adjuvant systemic therapy.		RS was not found to be predictive of distant disease recurrence.	

NSABP B–14 indicates National Surgical Adjuvant Breast and Bowel Project Protocol B–14; ER, estrogen receptor; RT–PCR, reverse transcriptate-polymerase chain reaction; RS, recurrence score.

ability was stronger in the patients with lymph node-negative disease. In the subgroup of patients with ER-positive, lymph node-negative disease, multivariate analysis including age, progesterone receptor status, tumor size, S-phase fraction, and tamoxifen treatment demonstrated the 2-gene ratio to be a significant predictor of RFS (HR, 3.9; 95% CI, 1.5–10.3 [$P = .007$]). As was noted in the NCCTG validation study,[16] the ratio was a better indicator of prognosis in the patients with lymph node-negative disease than in those with lymph node-positive disease. Although to our knowledge the mechanism for this disparity is unclear, the authors noted that lymph node-positive tumors tend to have a higher HOXB13:IL17BR expression ratio than lymph node-negative tumors.[17]

Jansen et al evaluated the HOXB13:IL17BR expression ratio in 1,252 patients with operable breast cancer and demonstrated that the ratio was associated with both tumor aggressiveness as well as the likelihood of tamoxifen failure.[18] A total of 468 patients with ER-positive, primary breast cancer were analyzed, 217 (46%) of whom relapsed during the follow-up period. The HOXB13:IL17BR expression ratio was found to be significantly associated with a poor DFS and OS. Expression levels were also evaluated in 193 patients with ER-positive, primary breast cancer who relapsed

and were treated with first-line tamoxifen therapy. The HOXB13:IL17BR expression ratio was found to be significantly associated with a poor response to tamoxifen ($P = .027$) and a short progression-free-survival ($P < .001$).

The HOXB13:IL17BR (H/I) index uses formalin-fixed, paraffin-embedded tissue and is commercially available in the United States.

The 21-Gene RT–PCR Assay (Onco*type* DX)

Prognostic Data

Table 2 shows prognostic data.

Although the signatures based on DNA arrays (eg, the 70-gene assay) have prognostic value, their clinical applicability has been limited by the need for fresh-frozen tissue. In an attempt to circumvent this issue, Cronin et al developed a real-time RT-PCR method to quantify gene expression in sections of fixed, paraffin-embedded tumor tissue.[19] By using the published literature and genomic databases, as well as experiments based on DNA arrays in fresh-frozen tissue, 250 candidate genes were selected[3,8,20,21] and their correlation with breast cancer recurrence was examined in 3 independent clinical breast cancer trials with a combined total of 447 patients.[22,23] Samples from these 3 trials were used to select a panel of 16 cancer-related and 5 reference genes (Table 3),[24–26] and an algorithm based

TABLE 3. Panel of 21 Genes

PROLIFERATION GENES	INVASION GENES	HER2	ESTROGEN	OTHER CANCER-RELATED GENES	REFERENCE GENES
Ki-67 STK15 Survivin CCNB1 (cyclin B1) MYBL2	MMP11 (stromolysin 3) CTSL2 (cathepsin L2)	GRB7 HER2	ER PR BCL2 SCUBE2	GSTM1 CD68 BAG1	ACTB (β-actin) GAPDH RPLPO GUS TFRC

MYBL2 indicates v-myb myeloblastosis viral oncogene homolog (avian)-like 2; MMP11, matrix metalloproteinase 11; GRB7, growth factor receptor-bound protein 7; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; GSTM1, glutathione S-transferase Mu 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RPLPO, ribosomal large protein; TFRC, transferrin receptor (p90, CD71).

on the expression of these genes was devised to compute a recurrence score (RS) for each tumor sample.

The 21-gene RT–PCR assay and the RS algorithm were validated in a population of patients with lymph node-negative disease who were treated with tamoxifen on a large, multicenter trial, the National Surgical Adjuvant Breast and Bowel Project Protocol B–14 (NSABP B–14) trial.[24] NSABP B–14 randomized 2,892 patients to receive either 5 years of treatment with tamoxifen or placebo and enrolled an additional 1,235 patients to receive an additional 5 years of tamoxifen treatment. Paraffin blocks with sufficient tumor tissue were available for 675 of the 2,617 patients treated with tamoxifen. RT–PCR was successful in 668 of the 675 samples. The expression levels of the 21 genes were used to calculate an RS and assign each patient to either a low-risk (RS <18), intermediate-risk (RS, 18–30), or high-risk (RS ≥31) group. The percentage of patients assigned to the low-risk, intermediate-risk, and high-risk RS groups were 51%, 22%, and 27%, respectively. The Kaplan–Meier estimates of the distant recurrence rate at 10 years were 6.8% (95% CI, 4.0–9.6%) in the low-risk group, 14.3% (95% CI, 8.3–20.3%) in the intermediate-risk group, and 30.5% (95% CI, 23.6–37.4%) in the high-risk group. The difference in the distant recurrence rate between the low-risk and high-risk groups was statistically significant ($P < .001$). The RS was also found to be predictive of OS ($P < .001$). In a multivariate Cox model, the RS was found to be a significant predictor of distant recurrence independent of age and tumor size ($P < .001$).

The results from the NSABP B–14 trial were independently confirmed in a community hospital setting.[25] A case-control study was performed among 4,964 patients from Kaiser Permanente who were diagnosed between 1985 and 1994 and not treated with adjuvant chemotherapy. The 220 cases were patients who died from breast cancer and the 570 controls were patients with breast cancer who were individually matched to cases with regard to age, race, adjuvant tamoxifen use, medical facility, and year of diagnosis and who were alive at the date of death of their matched case. After adjustment for grade and tumor size, the RS was found to be associated with the risk of breast cancer death in patients with ER-positive disease who were treated and those not treated with tamoxifen. The risks of death from breast cancer at 10 years in the patients treated with tamoxifen for the low-risk, intermediate-risk, and high-risk groups were 2.8% (95% CI, 1.7–3.9%), 10.7% (95% CI, 6.3–14.9%), and 15.5% (95% CI, 7.6–22.8%), respectively. In the patients not treated with tamoxifen, these risks were 6.2% (95% CI, 4.5–7.9%), 17.8% (95% CI, 11.8–23.3%), and 19.9% (95% CI, 14.2–25.2%), respectively. As was observed in the NSABP B–14 trial, approximately half of the patients had a low-risk RS.

We believe that the data presented above validate the use of the 21-gene assay in patients with ER-positive, lymph node-negative disease. Esteva et al evaluated the assay in a population of patients with lymph node-negative breast cancer who were treated at The University of Texas M. D. Anderson Cancer Center and who did not receive adjuvant chemotherapy and had been followed for a minimum of 5 years.[26] Of the 149 eligible patients, 69% had tumors that were ER positive. In this mixed group of patients, in terms of hormonal status, the RS was not found to be predictive of distant disease recurrence.

Predictive Data

The ability of gene expression profiling assays to predict benefit from chemotherapy in the neoadjuvant as well as adjuvant setting also has been evaluated (Table 4). Gianni et al evaluated the assay in 89 patients receiving neoadjuvant chemotherapy with three 3-week cycles of

TABLE 4. Validation Studies for Predictive Value of the 21-Gene Recurrence Score Assay

STUDY	POPULATION	RESULTS
Gianni 2005[27]	89 patients with locally advanced breast cancer who were receiving neoadjuvant paclitaxel and doxorubicin.	RS was positively associated with the likelihood of achieving a pCR (*P* = .005).
NSABP B-20 (Paik 2006)[28]	2,299 women with ER-positive, lymph node-negative breast cancer randomized to receive tamoxifen alone vs tamoxifen + MF vs tamoxifen + CMF. Gene expression results were available in 651 patients.	Significant interaction between RS and chemotherapy benefit. High RS: RR, 0.26; mean absolute decrease in 10-year distant recurrence rate, 27.6% Low RS: RR, 1.31; mean absolute decrease in 10-year distant recurrence rate, 1.1% Estimates in the intermediate RS group were too uncertain to exclude a clinically significant benefit.

RS indicates recurrence score; pCR, pathologic complete response; NSABP B-20, National Surgical Adjuvant Breast and Bowel Project B-20 trial; ER, estrogen receptor; MF, methotrexate and fluorouracil; CMF, cyclophosphamide, methotrexate, and fluorouracil; RR, relative risk.

doxorubicin (60 mg/m^2) and paclitaxel (200 mg/m^2) followed by 12 weeks of weekly paclitaxel (80 mg/m^2) (Table 4).[27] Adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) were administered after surgery.[27] RNA was extracted from pretreatment, formalin-fixed, paraffin-embedded core needle biopsies. Using RT–PCR, the expression of 384 genes was quantified and correlated with pCR. Eighty-six genes were found to correlate with a pCR and a pCR was more likely to occur with a higher expression of proliferation-related and immune-related genes and with a lower expression of ER-related genes. The RS, calculated from the 21-gene assay, was found to be positively associated with the likelihood of achieving a pCR (*P* = .005).[27]

The 21-gene assay has been evaluated for its ability to predict adjuvant chemotherapy benefit using the NSABP B–20 trial (Table 4).[28] The NSABP B–20 trial evaluated the benefit of adding CMF or methotrexate and fluorouracil to treatment with 5 years of tamoxifen in women with lymph node-negative, ER-positive breast cancer. Of 2,299 eligible patients, blocks with sufficient tumor tissue were available for 670 patients, from which gene expression results were obtained in 651. There was a significant interaction noted between RS and benefit from chemotherapy. Patients with a high RS were found to have a large benefit from chemotherapy (relative risk [RR], 0.26; 95% CI, 0.13−0.53 [mean absolute decrease in the 10-year distant recurrence rate, 27.6%]) whereas patients with a low RS derived little or no benefit (RR, 1.31; 95% CI, 0.46−3.78 [mean absolute decrease in the 10-year distant recurrence rate, 1.1%]). Although the patients with an intermediate

RS did not appear to have a large benefit, the estimates were too uncertain to exclude a clinically significant benefit.

Lymph Node-Positive Patients and Patients Treated with an Aromatase Inhibitor

To our knowledge, the 21-gene assay has been most extensively validated in women with lymph node-negative disease who were treated with tamoxifen. However, data are emerging regarding the prognostic and predictive value of the assay in women with lymph node-positive disease and in women treated with an aromatase inhibitor (AI). Goldstein et al[29] evaluated the prognostic value of the assay in a group of patients with ER-positive, early stage breast cancer, all of whom received adjuvant chemotherapy. The Eastern Cooperative Oncology Group (ECOG) study E2197 randomized 2,885 patient with operable breast cancer and 0 to 3 positive lymph nodes to four 3-week cycles of doxorubicin (60 mg/m^2) plus cyclophosphamide (600 mg/m^2) or docetaxel (60 mg/m^2) plus cyclophosphamide (600 mg/m^2). After chemotherapy, the patients with ER-positive disease received 5 years of tamoxifen treatment, although the trial was subsequently amended to allow the use of AIs. With a median follow-up of 76 months, there was no significant difference noted between the 2 treatment arms with regard to either DFS or OS.[29] A sample of 465 patients with ER-positive tumors with available tissue underwent the 21-gene assay, and also had their recurrence risk estimated by Adjuvant! Online.[30] The 5-year recurrence estimates were com-

puted by Adjuvant! Online and patients were classified as being at low, intermediate, or high risk using the previously defined criteria. The prognostic utility of RS was evaluated for each Adjuvant! Online risk group.

Similar to data previously reported in patients with lymph node-negative disease, 46% of the patients had a low-risk RS, 30% had an intermediate-risk RS, and 24% had a high-risk RS. The RS was found to be a highly significant predictor of local as well as distant recurrence in both the patients with lymph node-negative ($P = .0007$) and lymph node-positive ($P = .0004$) disease. Furthermore, a low RS predicted a low risk of recurrence ($\leq 5\%$), irrespective of lymph node status. The RS provided additional prognostic information to Adjuvant! Online, particularly with regard to those patients projected to have better outcomes.

All the patients in the ECOG study E2197 received chemotherapy. However, Albain et al evaluated the 21-gene assay in patients with lymph node-positive, ER-positive disease who were treated with adjuvant tamoxifen alone.[31] The Southwest Oncology Group Intergroup Trial S8814 was a phase 3 trial of postmenopausal women with lymph node-positive, ER-positive breast cancer that demonstrated that the addition of 6 cycles of cyclophosphamide, doxorubicin, and fluorouracil (CAF) added a significant benefit with regard to DFS and OS compared with tamoxifen alone, particularly if CAF and tamoxifen were administered sequentially (CAF–T). Of the 927 patients randomized to receive either tamoxifen alone or CAF–T, 45% provided specimens, with 367 patients (148 treated with tamoxifen alone and 219 treated with CAF–T) found to have sufficient RNA for RT–PCR analysis. The RS risk distribution was somewhat different from that noted in patients with lymph node-negative disease: 40% in the low-risk group, 28% in the intermediate-risk group, and 32% in the high-risk group. The RS was found to be prognostic for DFS and OS in the patients treated with tamoxifen alone ($P = .006$). There was a large benefit noted for CAF–T compared with tamoxifen alone in the high-risk RS subset but no apparent benefit was observed in the low-risk RS group. The 10-year DFS estimates (95% CI) were 60% for tamoxifen alone versus 64% for CAF–T in the low-risk group, 49% for tamoxifen alone versus 63% for CAF–T in the intermediate-

risk group, and 43% for tamoxifen alone versus 55% for CAF–T in the high-risk group.

The majority of the data regarding the 21-gene assay in patients with ER-positive disease have been derived from patients treated with tamoxifen. However, currently, tamoxifen is not the only drug available for the adjuvant treatment of postmenopausal women with early stage breast cancer and many patients are in fact receiving an AI instead. The ability of the 21-gene assay to predict the risk of distant recurrence in postmenopausal women receiving an adjuvant AI has been evaluated in the TransATAC analysis of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial.[32] The ATAC trial randomized 9,366 patients with early stage breast cancer to 5 years of treatment with tamoxifen, 5 years of treatment with anastrazole, or 5 years of treatment with both tamoxifen and anastrazole. Of the 9,366 women, 3,486 were either negative for ER or were randomized to the combination arm and therefore were not included in the TransATAC analysis. Of the remaining 5,880 patients who were eligible for the TransATAC analysis, blocks were available with sufficient tumor in 1,856 patients and a reportable RS was obtained in 1,308 patients, of whom 1,231 were evaluable. In the prospectively defined, primary, multivariate analysis, tumor size, tumor grade, and RS were each found to be separately statistically significant in predicting time to distant recurrence in patients with lymph node-negative disease ($P < .001$, $P = .003$, and $P < .001$, respectively) with similar results observed in patients with lymph node-positive disease. For the patients with lymph node-negative disease, the 9-year distant recurrence rates for the low-risk, intermediate-risk, and high-risk RS groups were 4%, 12%, and 25%, respectively; and those for the patients with lymph node-positive disease were 17%, 28%, and 49%, respectively. The RS demonstrated statistically significant prognostic value beyond that provided by Adjuvant! Online with regard to both lymph node-negative ($P < . 001$) and lymph node-positive patients ($P = .003$). The data were not predictive of a differential benefit between tamoxifen and anastrazole.

Comparison of Gene Expression Assays

Several of the gene expression assays discussed have been compared. Fan et al used 295 samples to com-

pare predictions from 5 gene expression assays: intrinsic subtypes, the 70-gene profile, the 2-gene HOXB13:IL17BR expression ratio, the 21-gene RS assay, and the wound response assay.[33] The wound response signature, identified by testing the correlation between tumor progression and a gene expression program identified in an experimental wound response model, was previously validated in 295 consecutive patients with early stage breast cancer.[34,35] Despite the absence of gene overlap, the assays, with the exception of the HOXB13:IL17BR expression ratio, demonstrated high concordance rates in predicting outcome, suggesting that the assays identify common biologic characteristics that are predictive of patient outcomes.

Cost-Effectiveness of the Assays

The emergence of commercially available gene profiling assays has raised the question of the cost-effectiveness of these techniques. Cost-effectiveness analyses comparing 2 of the commercially available assays in the United States, the 21-gene RS assay and the 70-gene assay, with other methods of assessment have been performed recently.

A cost-utility analysis was conducted using the 21-gene RS assay in patients previously classified as having a low or high risk of distant recurrence based on clinical guidelines published by the National Comprehensive Cancer Network (NCCN).[36] The cost of the assay was estimated at $3,460. The analysis demonstrated that using the assay to guide chemotherapy decisions provided a net savings of $2,256 compared with chemotherapy and tamoxifen, with an incremental cost-effectiveness ratio of $1,944 per life saved with treatment with tamoxifen alone. Furthermore, using the assay to guide therapy was associated with a gain in individual life expectancy of 2.2 years compared with tamoxifen alone and a similar life expectancy compared with the use of tamoxifen and chemotherapy.

A similar analysis estimating the costs and cost-effectiveness of the 70-gene assay versus Adjuvant! Online in deciding whether to use adjuvant chemotherapy for women aged 61 years and younger with lymph node-negative, HER2-negative, early stage breast cancer with ER-positive or negative status was recently reported.[37] Compared with Adjuvant! Online, using the 70-gene assay resulted in 35% of patients being reassigned to a different risk classification and chemotherapy was avoided in 9% of the patients. In the base case, the 70-gene signature strategy was found to be cost-neutral. Lifetime costs per patient were $178,811 and $178,893, respectively, for the 70-gene assay and Adjuvant! Online strategies. Use of the 70-gene assay was associated with an increase of 0.13 life-years and 0.16 quality-adjusted life-years.

Current Use of the Assays

Currently, the 70-gene assay and the 21-gene RS assay are the most commonly used genomic profiling assays in Europe and the United States. MammaPrint was the first assay in the United States to receive US Food and Drug Administration (FDA) approval under the FDA's new, in vitro, diagnostic, multivariate index assay classification as a prognostic test for women aged younger than 61 years with ER-positive or ER-negative, lymph node-negative breast cancer. Onco*type* DX has been exempt from this approval process.

In the United States, Onco*type* DX is currently the most commonly used assay in clinical practice for a variety of reasons, including the finding that it can be performed on formalin-fixed, paraffin-embedded tissue. It is unclear at the present time how the need for fresh tissue will affect the adoption of MammaPrint in the United States. In addition, the use of the Onco*type* DX assay to predict the risk of recurrence and the benefits of tamoxifen and CMF chemotherapy in newly diagnosed patients with lymph node-negative, ER-positive breast cancer are included in the 2007 American Society of Clinical Oncology (ASCO) tumor marker guidelines.[38] The ASCO panel believed that the precise clinical utility and appropriate application of other assays (eg, the 70-gene or 76-gene assays) were "under investigation."

However, there are limitations to the use of the Onco*type* DX assay, including the lack of a data-driven answer regarding the optimal treatment of patients with an intermediate-risk RS. In addition, use of the Onco*type* DX assay is limited to patients with ER-positive disease, unlike other assays (including the MammaPrint assay), which have been validated in patients with both ER-positive and ER-negative disease. Currently, both assays cost approximately $3,000 to $4,000 in the United States.

Future Directions

As a result of gene expression assays, there is growing recognition that breast cancer is a molecularly heterogeneous disease. However, there are multiple unresolved issues with regard to the adoption of these assays. For example, there are very few data regarding the biologic reproducibility of these assays; the effect of variable tumor cellularity as well as intratumoral heterogeneity on the molecular classification; and the potential for contamination by normal breast tissue or in situ carcinomas, particularly in small invasive tumors. Similarly, the effect of a prior biopsy on gene expression results has to our knowledge been underexplored. Furthermore, the usefulness of these assays in other clinical settings (eg, in patients with locally advanced or metastatic breast cancer) has not been adequately examined.

We await further data to clarify the optimal use of these assays, particularly prospective, randomized data. In Europe, the 70-gene assay is currently being evaluated in a prospective clinical fashion in the European Organization for Research and Treatment of Cancer (EORTC) Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy (MINDACT) trial.[39] This trial aims to enroll 6,000 patients with lymph node-negative breast cancer who will have their risk assessed both by Adjuvant! Online and the 70-gene profile. If both methodologies assess the patient as having a low relapse risk, no chemotherapy will be administered. If both methods classify the relapse risk as high, chemotherapy will be administered. If the methods are discordant, the patient will be randomly assigned to follow the results of Adjuvant! Online or the 70-gene assay.[39] In North America, the 21-gene assay is currently being evaluated in a prospective clinical fashion in the Trial Assigning IndividuaLized Options for Treatment (Rx) (TAILORx) trial. Patients with lymph node-negative, ER-positive breast cancer will be divided into 3 treatment arms depending on their RS. However, the RS categories are different from those previously validated. A low-risk RS on the TAILORx trial is <11, an intermediate-risk RS is between 11 and 25, and a high-risk RS is >25. The purpose of these adjustments was to minimize the potential for undertreatment in the intermediate-risk and high-risk groups.[40] Patients with a low-risk RS will receive endocrine therapy without chemotherapy, patients with a high-risk RS will receive chemotherapy followed by endocrine therapy, and patients in the intermediate-risk RS category will be randomized to receive either endocrine therapy without chemotherapy or chemotherapy followed by endocrine therapy. The choice of chemotherapy regimen and endocrine therapy (tamoxifen or an AI) will be at the discretion of the treating physician. Exclusion criteria include HER2-positive tumors. These trials, along with the incorporation of tissue collection and genomic profiling into general clinical trial design, will improve our ability to optimally tailor therapy for individual patients. ■

References

1. Ravdin PM, Siminoff LA, Davis GJ, et al. Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J Clin Oncol.* 2001;29:980–991.

2. Olivotto IA, Bajdik CD, Ravdin PM, et al. Population-based validation of the prognostic model ADJUVANT! for early stage breast cancer. *J Clin Oncol.* 2005;23:2716–2725.

3. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature.* 2000;406:747–752.

4. Sorlie T, Perou AM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A.* 2001;98:10869–10874.

5. Yu K, Lee CH, Tan PH, et al. Conservation of breast cancer molecular subtypes and transcriptional patterns of tumor progression across distinct ethnic populations. *Clin Cancer Res.* 2004;10:5508–5517.

6. Weigelt B, Hu Z, He X, et al. Molecular portraits and 70-gene prognosis signature are preserved throughout the metastatic process of breast cancer. *Cancer Res.* 2005; 65:9155–9158.

7. Parker JS, Mullins M, Cheang MCU, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27:1160–1167.

8. van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature.* 2002;415:530–536.

9. van de Vijver, He Y, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med.* 2002;347:1999–2009.

10. Buyse M, Loi S, van't Veer L, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst.* 2006;98:1183–1192.

11. Bender RA, Knauer M, Rutgers EJ, et al. The 70-gene profile and chemotherapy benefit in 1600 breast cancer patients [abstract]. *J Clin Oncol.* 2009;27(suppl):15s. Abstract 512.

12. Wang Y, Klijn JGM, Sieuwerts AM, et al. Gene-expression profiles to predict distant metastasis of lymph node-negative primary breast cancer. *Lancet.* 2005;365:671–679.

13. Foekens JA, Atkins D, Zhang Yi, et al. Multicenter validation of a gene expression-based prognostic signature in lymph node-negative primary breast cancer. *J Clin Oncol.* 2006;24:1665–1671.

14. Ma XJ, Wang Z, Ryan PD, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell.* 2004;5:607–616.

15. Jerevall PL, Brommesson S, Strand C, et al. Exploring the two-gene ratio in breast cancer-independent roles for HOXB13 and IL 17BR in prediction of clinical outcome. *Breast Cancer Res Treat*. 2008;107:225–234.

16. Goetz MP, Suman VJ, Ingle JN, et al. A two-gene expression ratio of homeobox 13 and interleukin–17B receptor for prediction of recurrence and survival in women receiving adjuvant tamoxifen. *Clin Cancer Res*. 2006;12:2080–2087.

17. Ma XJ, Hilsenback SG, Wang W, et al. The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer. *J Clin Oncol*. 2006;24:4611–4617.

18. Jansen MP, Siewerts AM, Look MP, et al. HOXB13–to–IL17BR expression ratio is related to tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study. *J Clin Oncol*. 2007;25:662–668.

19. Cronin M, Pho M, Dutta D, et al. Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. *Am J Pathol*. 2004;164:35–42.

20. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*. 1999;286:531–537.

21. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001;98:10869–10874.

22. Paik S, Shak S, Tang G, et al. Multi-gene RT–PCR assay for predicting recurrence in node negative breast cancer patients—NSABP studies B–20 and B–14 [abstract]. *Proc Am Soc Clin Oncol*. 2003;22:850.

23. Cobleigh MA, Tabesh B, Bitterman P, et al. Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes. *Clin Cancer Res*. 2005;11:8623–8631.

24. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351:2817–2826.

25. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res*. 2006;8:1–15.

26. Esteva FJ, Sahin AA, Cristofanilli M, et al. Prognostic role of a multigene reverse transcriptase-PCR assay in patients with node-negative breast cancer not receiving adjuvant systemic therapy. *Clin Cancer Res*. 2005;11:3315–3319.

27. Gianni L, Zambetti M, Clark K, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol*. 2005;23:7265–7277.

28. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006;24:3726–3734.

29. Goldstein LJ, O'Neill A, Sparano JA, et al. Concurrent doxorubicin plus docetaxel is not more effective than concurrent doxorubicin plus cyclophosphamide in operable breast cancer with 0 to 3 positive axillary nodes: North American Breast Cancer Intergroup Trial E 2197. *J Clin Oncol*. 2008;26:4092–4099.

30. Goldstein L, Gray R, Badve S, et al. Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinico-pathologic features. *J Clin Oncol*. 2008;26:4063–4071.

31. Albain K, Barlow W, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal, node-positive, ER-positive breast cancer (S8814, INT0100) [abstract]. *Br Cancer Res Treat* 2007;106(suppl 1). Abstract 10.

32. Dowsett M, Cuzick J, Wales C, et al. Risk of distant recurrence using oncotype DX in postmenopausal primary breast cancer patients treated with anastrozole or tamoxifen: a TransATAC study [abstract]. *Cancer Res* 2009;69(suppl). Abstract 53.

33. Fan C, Oh DS, Wessels L, et al. Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med*. 2006;355:560–569.

34. Chang HY, Sneddon JB, Alizadeh AA, et al. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. *PLoS Biol*. 2004;2:E7.

35. Chang HY, Nuyten DSA, Sneddon JB, et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. *Proc Natl Acad Sci U S A*. 2005;102:3738–3743.

36. Lyman GH, Cosler LE, Kuderer NM, et al. Impact of a 21-gene RT–PCR assay on treatment decisions in early-stage breast cancer: an economic analysis based on prognostic and predictive validation studies. *Cancer*. 2007;109:1011–1018.

37. Tong KB, Chen E, Brink G, et al. Cost-effectiveness of targeting chemotherapy with the 70-gene prognostic signature in early-stage breast cancer (ESBC) patients [abstract]. *J Clin Oncol*. 2009;27(suppl):15s. Abstract 6570.

38. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007;25:5287–5312.

39. Cardoso F, van't Veer L, Rutgers E, et al. Clinical application of the 70-gene profile: the MINDACT trial. *J Clin Oncol*. 2008;26:729–735.

40. Sparano JA, Paik S. Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol*. 2008;26:721–728.