

## Development of the 21-Gene Assay and Its Application in Clinical Practice and Clinical Trials

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### ABSTRACT

Several multigene markers that predict relapse more accurately than classical clinicopathologic features have been developed. The 21-gene assay was developed specifically for patients with estrogen receptor (ER)-positive breast cancer, and has been shown to predict distant recurrence more accurately than classical clinicopathologic features in patients with ER-positive breast cancer and negative axillary nodes treated with adjuvant tamoxifen; validation studies in this population led to its approval as a diagnostic test. In a similar population, it also may be used to assess the benefit of adding chemotherapy to hormonal therapy. Other validation studies indicate that it also predicts the risk of distant and local recurrence in other populations with ER-positive disease, including node-negative patients receiving no adjuvant therapy and patients with positive axillary nodes treated with doxorubicin-containing chemotherapy. The Trial Assigning Individualized Options for Treatment (TAILORx) is multicenter trial that integrates the 21-gene assay into the clinical decision-making process and is designed to refine the utility of the assay in clinical practice and to provide a resource for evaluating additional molecular markers as they are developed in the future.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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### INTRODUCTION

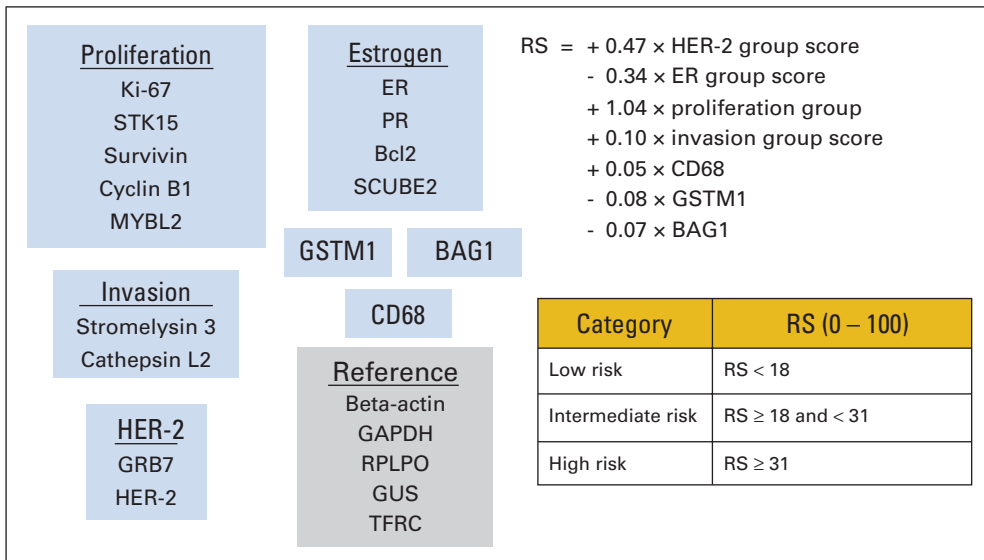
There have been substantial technical and analytic advances within the last decade that have facilitated high-throughput analysis of clinical specimens for genomic expression patterns, a process that has been referred to as “genomic profiling.”<sup>1,2</sup> Comparing the genomic expression patterns of breast cancer specimens from relapsing with nonrelapsing patients has facilitated the development of multigene markers that predict clinical outcome more accurately than classical clinicopathologic features. The promise and pitfalls in developing such markers has been reviewed elsewhere,<sup>3-6</sup> and specific criteria have been proposed for the level of evidence required to define and support their clinical utility.<sup>7</sup>

Several multigene markers that were developed specifically for classifying prognosis have undergone external validation in independent data sets, the most stringent test for confirming the validity of a marker. These have included a 70-gene profile<sup>8,9</sup> a 76-gene profile<sup>10,11</sup> a 21-gene profile<sup>12,13</sup> a two-gene profile,<sup>14,15</sup> and a 97-genomic grade gene profile.<sup>16</sup> Other markers that were not developed for the purpose of classifying prognosis that have undergone external validation have been shown to predict outcomes, such as the intrinsic gene set<sup>17,18</sup> and the wound response signature.<sup>19,20</sup> These markers vary

considerably in the clinical contexts in which they were developed and validated. This review will focus on the 21-gene assay (Oncotype Dx; Genomic Health, Redwood City, CA), including studies that led to its development and validation, preliminary experience with its application in clinical practice, and trials designed to integrate the marker into clinical decision making.

### TRAINING SET STUDIES FOR THE DEVELOPMENT OF THE 21-GENE ASSAY

The 21-gene assay includes 16 tumor-associated genes and five reference genes, with the result expressed as a computed recurrence score (RS; Fig 1).<sup>12</sup> The process for selection of the genes and development of the RS algorithm, extracting RNA from formalin-fixed, paraffin-embedded tumor (FPET), performing real-time reverse transcriptase polymerase chain reaction (RT-PCR) in RNA extracted from PFET, and ensuring reproducibility of these procedures have been reviewed elsewhere.<sup>12,21,22</sup> Higher expression levels of “favorable” genes (estrogen receptor [ER] group, *GSTM1*, *BAG1*) results in a lower RS (because of a negative coefficient in the RS algorithm), whereas higher expression of “unfavorable” genes (proliferation group, human epidermal growth factor [HER]-2



**Fig 1.** Oncotype Dx (Genomic Health, Redwood City, CA) recurrence score (RS): genes and algorithm. HER, human epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor.

group, invasion group, and *CD68*) contribute to a higher RS (because of a positive coefficient in the RS algorithm).

#### PROSPECTIVE VALIDATION STUDY OF 21-GENE ASSAY

The 21-gene assay was subsequently evaluated in a prospective validation study that was performed in patients with ER-positive, node-negative breast cancer who were treated with tamoxifen in the National Surgical Adjuvant Breast and Bowel (NSABP) trial B-14.<sup>12</sup> The B-14 study included 2,644 patients with ER-positive breast cancer (defined as greater than or equal to 10 fmol/mg protein) and histologically negative axillary lymph nodes who were randomly assigned to tamoxifen (10 mg BID for 5 years or more depending on second random assignment) or placebo. Results from that trial demonstrated that tamoxifen significantly reduced the risk of recurrence (hazard ratio [HR] = 0.58;  $P < .0001$ ) and death (HR = 0.78;  $P = .0008$ ) through 15 years of follow-up irrespective of age, menopausal status, or ER concentration.<sup>23-25</sup> The 21-gene validation study included 668 of 675 patients for whom tumor blocks were available and for whom there was sufficient tumor for analysis. When evaluated as a categorical variable, the proportions of patients categorized as having a RS defined as low (< 18), intermediate,<sup>18-30</sup> or high ( $\geq 31$ ) by the RT-PCR assay were 51%, 22%, and 27%, respectively. The Kaplan-Meier estimates of the rates of distant recurrence at 5 and 10 years in the low-, intermediate-, and high-risk groups are shown in Table 1 and Figure 2A. RS was also predictive of distant recurrence at 10 years when evaluated as a continuous variable, as shown in Figure 2B. As shown in

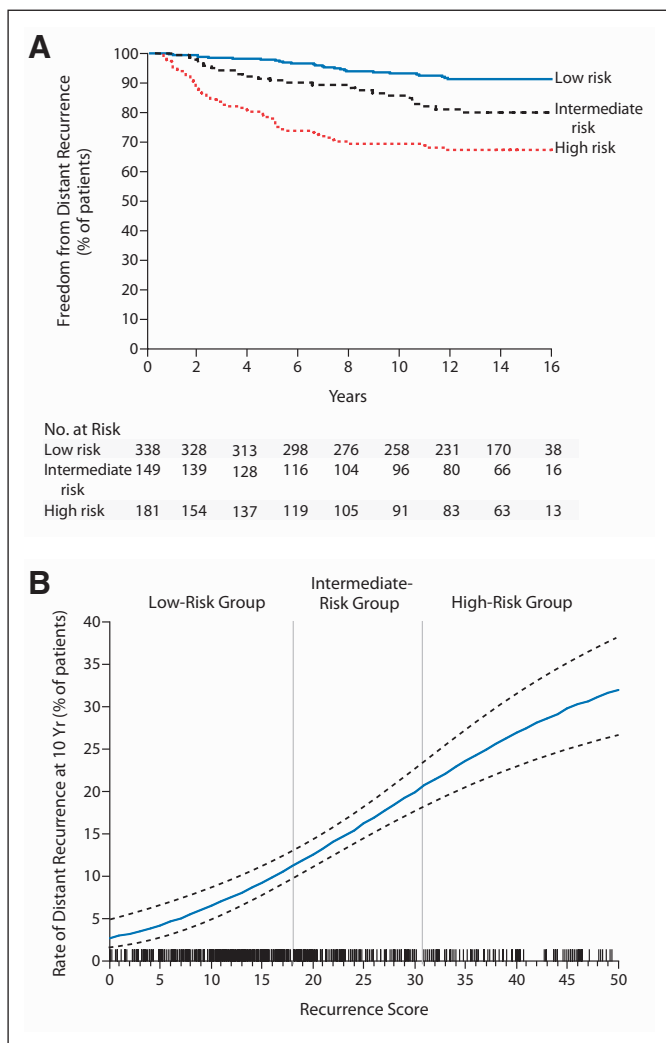
Table 1, there were significant differences in the frequency of distant recurrence among the three groups at 10 years, but also in the timing of recurrence; approximately two thirds of recurring tumors with an intermediate or high RS recurred within 5 years, compared with only one third of recurring tumors with a low RS. In a multivariate Cox model, RS predicted distant recurrence independent of age and tumor size ( $P < .001$ ), and was also predictive of overall survival ( $P < .001$ ). Another analysis of this data set indicated that RS more accurately predicted recurrence than Adjuvant! (www.adjuvantonline.com), an externally validated integrator of clinical and treatment information that is commonly used in clinical practice,<sup>26</sup> providing further evidence that it was more accurate in predicting clinical outcome than classical clinicopathologic features.<sup>27</sup>

#### POPULATION-BASED EXTERNAL VALIDATION STUDY

A population-based external validation study reported by Habel et al<sup>13</sup> performed in a separate group of patients with similar characteristics confirmed the findings of the B-14 validation study. The report described a case-control study conducted among 4,964 patients diagnosed with node-negative invasive breast cancer who did not receive adjuvant chemotherapy. The analysis included 220 patients who died as a result of breast cancer and 570 controls who were individually matched to patients with respect to age, race, adjuvant tamoxifen, medical facility, and diagnosis year, and who were alive at the date of death of their matched patient. Tumor blocks were retrieved for analysis, of which approximately 50% had a low RS in both the

**Table 1.** Risk of Distant Recurrence at 5 and 10 Years by Recurrence Score in the B14 Validation Study

Recurrence Score	Risk Group	No. of Patients	%	10-Year Distant Recurrence (%)		5-Year Distant Recurrence (%)	
				Rate	95% CI	Rate	95% CI
< 18	Low	338	51	6.8	4 to 9.6	2.1	0.6 to 3.7
18-30	Intermediate	149	22	14.3	8.3 to 20.3	9.2	4.4 to 14
$\geq 31$	High	181	27	30.5	23.6 to 37.4	22.1	15.9 to 28.2



**Fig 2.** Oncotype Dx (Genomic Health, Redwood City, CA) recurrence score predicts distant recurrence when analyzed as either a (A) categoric or (B) continuous variable. The continuous function in Part B was generated with use of a piecewise log-hazard ratio model. The dashed curves indicate the 95% CI. The rug plot on top of the x-axis shows the recurrence score for individual patients in the study. Reprinted with permission from Paik S, Shak S, Tang G, et al: N Engl J Med 351:2817-2826, 2004.<sup>12</sup>

tamoxifen-treated and untreated groups. After adjusting for tumor size and grade, the RS was associated with the risk of breast cancer death in ER-positive, tamoxifen-treated, and untreated patients ( $P = .003$  and  $.03$ , respectively). At 10 years, the risks for breast cancer death in tamoxifen-treated patients were 2.8% (95% CI, 1.7% to 3.9%), 10.7% (95% CI, 6.3% to 14.9%), and 15.5% (95% CI, 7.6% to 22.8%) for those in the low, intermediate, and high RS groups, respectively. The strong correlation between RS and breast cancer death provided additional evidence for the robustness of the assay in this population. The results of the B-14 validation study and the Kaiser external validation study provided a sufficient level of evidence to support regulatory approval of Oncotype Dx as a diagnostic test by CLIA (under the provisions of the Clinical Laboratory Improvement Act of 1988)<sup>28</sup> for ER-positive, lymph node-negative breast cancer treated with tamoxifen. The analytic and operational performance specifications defined for the Oncotype Dx assay allow the reporting of quantitative RS

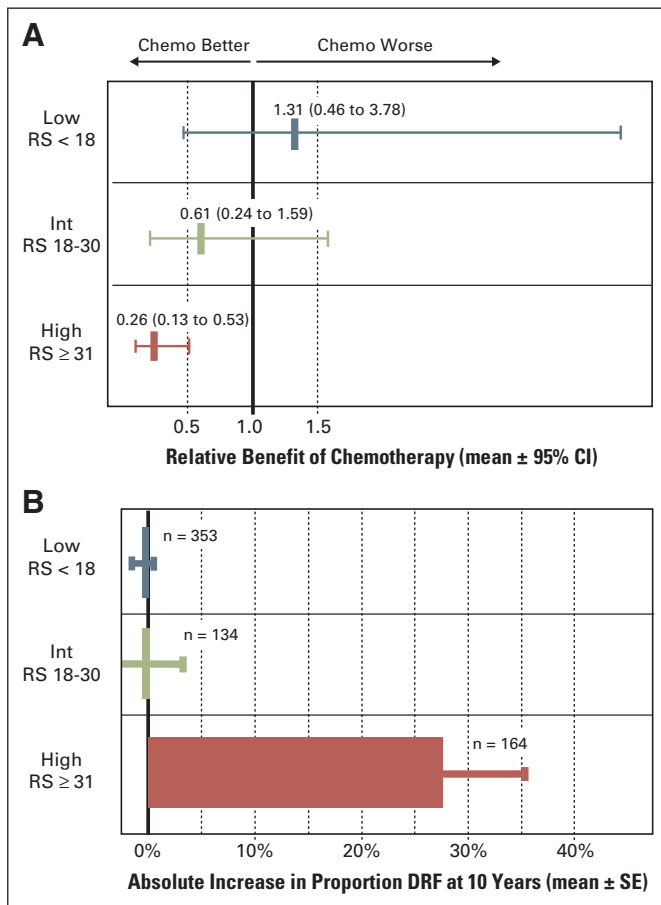
values for individual patients with a standard deviation of within 2 RS units on a 100-unit scale.<sup>22</sup>

**PROGNOSTIC VERSUS PREDICTIVE VALUE OF 21-GENE ASSAY FOR HORMONAL THERAPY**

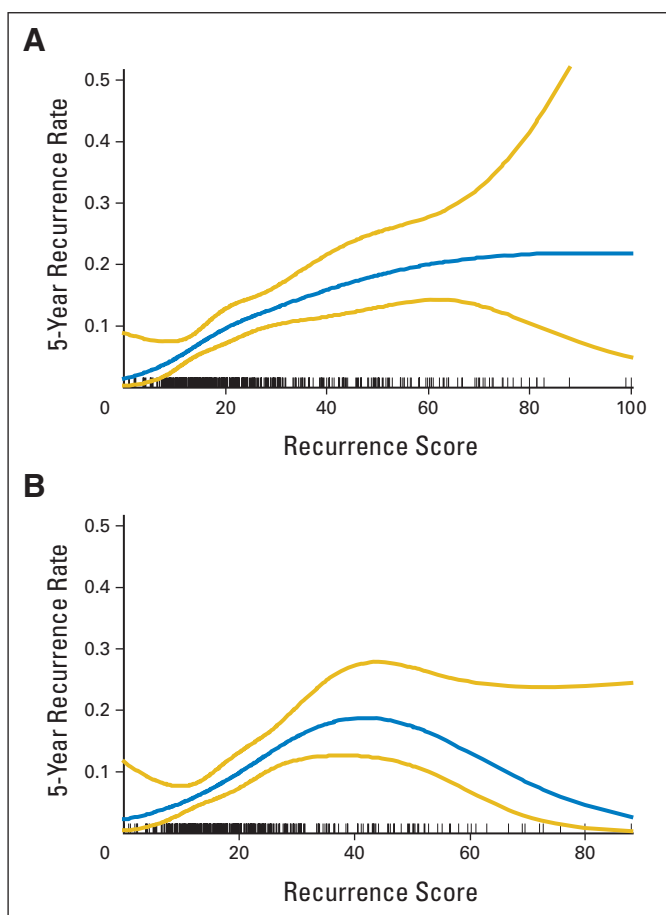
The 21-gene assay was also evaluated in the placebo arm of the B-14 trial, facilitating evaluation of its prognostic value in an untreated population, and evaluation of its role in predicting benefit from tamoxifen.<sup>29</sup> As in tamoxifen-treated patients, higher RS was associated with an elevated risk of recurrence whether evaluated as a categoric or continuous variable. Patients with a low ( $P = .02$ ) or intermediate ( $P = .04$ ) RS derived substantial benefit from tamoxifen, whereas patients with a high RS derived no benefit ( $P = .82$ ). Although the limited sample size precludes ruling out a benefit for tamoxifen in the high RS subset, the findings are consistent with the notion that lower RS predicts relatively greater benefit from tamoxifen.

**PROGNOSTIC VERSUS PREDICTIVE VALUE OF 21-GENE ASSAY FOR CHEMOTHERAPY**

Subsequent studies in patients treated with tamoxifen with or without adjuvant chemotherapy in NSABP trial B-20 indicated that high RS



**Fig 3.** (A) Relative and (B) absolute risk of chemotherapy (Chemo) benefit as a function of recurrence score (RS) risk category in low, intermediate (Int), and high recurrence score groups. Reprinted with permission from Paik S, Tang G, Shak S, et al: J Clin Oncol 24:3726-3734, 2006.<sup>31</sup> DRF, distant recurrence free.



**Fig 4.** Relationship between recurrence score (RS) and recurrence as a continuous variable modeled as a smooth continuous function using splines (blue line) with 95% CI, (purple lines) for all patients with (A) hormone receptor (HR)-positive disease and (B) HR-positive and human epidermal growth factor (HER)-negative disease where HER-positive disease was excluded. The rug plots above the x-axis show the individual patients with recurrence in blue and the individual patients without recurrence in yellow. The large CIs at higher RS reflect the relatively small number of patients with RS > 50.

seemed to predict benefit from adjuvant chemotherapy. The B-20 trial included 2,306 women with ER-positive, lymph node-negative breast cancer who were randomly assigned to received tamoxifen for 5 years either alone ( $n = 771$ ) or plus chemotherapy (including methotrexate-fluorouracil [MF;  $n = 767$ ], or cyclophosphamide, methotrexate, and fluorouracil [CMF;  $n = 768$ ]).<sup>30</sup> The addition of CMF to tamoxifen was associated with a lower risk of recurrence (HR = 0.52;  $P < .0001$ ) and death (HR = 0.78;  $P = .063$ ) through 12 years of follow-up, which translated into a 10% absolute improvement in recurrence-free survival and 4% absolute improvement in overall survival (with similar benefits seen for MF).<sup>24,25</sup> The 21-gene assay was

performed in a subset of 651 patients with tumor blocks available for analysis, including 424 randomly assigned to tamoxifen plus chemotherapy (CMF or MF) and 227 randomly assigned to tamoxifen (the latter of whom were used in the training set for the development of the 21-gene assay).<sup>31</sup> There was a large chemotherapy benefit if the RS was high (HR = 0.26; 95% CI, 0.13 to 0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; SE, 8.0%; Fig 3A-B). On the other hand, there was minimal, if any, benefit from chemotherapy if the RS was low (relative risk = 1.31; 95% CI, 0.46 to 3.78; absolute decrease in distant recurrence rate at 10 years: mean, -1.1%; SE, 2.2%). The test for interaction between chemotherapy treatment and RS was statistically significant ( $P = .038$ ). For those with an intermediate RS, chemotherapy did not seem to confer benefit, but uncertainty in the estimate could not exclude a clinically important benefit. Similar findings have been reported in another trial comparing tamoxifen with tamoxifen plus cyclophosphamide, doxorubicin, and fluorouracil chemotherapy in postmenopausal women with positive axillary nodes.<sup>32</sup>

#### OTHER VALIDATION STUDIES IN OTHER POPULATIONS

The 21-gene assay was also evaluated in a cohort of patients with operable breast cancer and up to three positive axillary nodes who participated in Intergroup trial E2197. The study included 2,885 eligible patients who were randomly assigned to receive four cycles of doxorubicin plus either cyclophosphamide (AC) or docetaxel (AT) every 3 weeks followed by hormonal therapy and irradiation when indicated. There was no difference in outcome between treatment arms after a median follow-up of 76 months. A sample of 776 patients who had tumor samples available, including all available samples from 178 patients who had a recurrence and a control group of 598 who had not recurred.<sup>33</sup> The RS was high in 98% of the 311 patients with hormone receptor-negative disease (defined as both ER and progesterone receptor [PR] negative), reflecting the fact that the assay was specifically developed for hormone receptor-positive disease. For the 465 patients with hormone receptor-positive disease (defined as ER and/or PR positive), RS was a highly significant predictor of recurrence, including node-negative and node-positive disease ( $P < .001$  for both). It was also a significant predictor of recurrence when adjusted for clinical variables, including tumor size, grade, age, HER2/neu expression, and nodal status. If the RS was less than 18, approximately 3.3% of patients (95% CI, 2.2% to 5.0%) experienced recurrence within 5 years if there were zero to one positive nodes, and 7.9% (95% CI, 4.3% to 14.1%) experienced recurrence if there were two to three positive nodes. When modeled as a smooth continuous function using splines, there was a direct correlation between RS and recurrence risk up to an RS of approximately 40 ( $P < .001$ ; Fig 4A),

**Table 2.** Studies Evaluating Impact of 21-Gene Assay on Decision Making by Community and/or Academic Medical Oncologists

Author	No. of Patients	No. of Oncologists	Setting	Change in Treatment Recommendation (%)
Lo et al <sup>41</sup>	89	15	Community and academic	26
Oratz et al <sup>42</sup>	68	4	Community	20
Kamal et al <sup>43</sup>	31	5	Academic	18

even if the analysis was restricted to only patients with *HER2*-negative disease ( $P < .001$ ; Fig 4B). Risk of recurrence did not increase higher than an RS of 40 in this group of patients treated with chemotherapy, which was consistent with previous reports suggesting greater benefit from chemotherapy if the RS was high,<sup>34</sup> but also may have reflected the effect of a few influential cases in the relatively small number of patients with an elevated RS in this analysis.

The 21-gene assay has also been shown to predict pathologic complete response to preoperative chemotherapy,<sup>35</sup> and other multi-gene signatures have been shown to predict response to preoperative therapy.<sup>36,37</sup> There are several ongoing studies that will shed further light on the clinical utility of the 21-gene assay, including analyses evaluating its prognostic utility in patients receiving aromatase inhibitor therapy and other populations.

#### CONTRIBUTION OF GENE GROUPS AND INDIVIDUAL GENES IN THE 21-GENE ASSAY

The 21-gene assay uses an RT-PCR–based assay rather than conventional immunohistochemistry (IHC), and the RS algorithm is weighted heavily toward genes involved in ER signaling, proliferation, and *HER2* expression. This raises the possibility that the greater accuracy of the assay in predicting outcomes might be solely attributed to more accurate quantitation of ER expression, or a combination of ER expression and proliferation.

With regard to the ER expression, Kim et al<sup>38</sup> compared ER expression by ligand binding (LB) assay (performed by the study sites at enrollment), RT-PCR (performed in the Genomic Health laboratory), and IHC (performed by the NSABP laboratory). There was fair correlation of LB with either IHC or RT-PCR (Pearson  $r = 0.3$  and  $0.4$ , respectively), and moderate correlation for IHC with RT-PCR ( $r = 0.5$ ). The HRs for the association of ER measured by all three methods and distant relapse–free interval (DRFI) were 0.86 for LB (95% CI, 0.70 to 1.08;  $P = .191$ ), 0.44 for IHC (95% CI, 0.21 to 0.89;  $P = .023$ ), and 0.14 for RT-PCR (95% CI, 0.07 to 0.29;  $P < .001$ ), indicating that the quantitative RT-PCR measure of ER expression correlated with sensitivity to tamoxifen therapy.

With regard to ER expression, its relationship to RS, and contribution of other genes, Paik<sup>29</sup> has pointed out that tumors that had high ER expression in the B-14 and B-20 study may have either a low or high RS. To further investigate the contribution of other genes, the relationship between ER expression and proliferation and *HER2* genes

were evaluated in 10,618 breast cancers by the Genomic Health laboratory in their commercial experience.<sup>39</sup> The proliferation index (PI) was found to be largely independent of ER and PR expression. The PI was 1.6-fold higher on average in *HER2*-positive compared with *HER2*-negative tumors, but could be high or low for any level of *HER2* expression; *HER2* expression accounted for less than 2% of the variance in the PI. Regarding all genes in the assay, ranges of expression from 32- to more than 2,000-fold were observed.<sup>40</sup> Conditional on ranges of expression, any single gene or gene group altered the RS by much as 5 to 59 RS units, indicating that genes other than those involved in ER signaling, proliferation, and *HER2* that had smaller coefficients and wide ranges of expression influenced the RS in a clinically significant way, especially for low-risk patients.

#### POSTMARKETING EXPERIENCE AND IMPACT ON CLINICAL DECISION MAKING

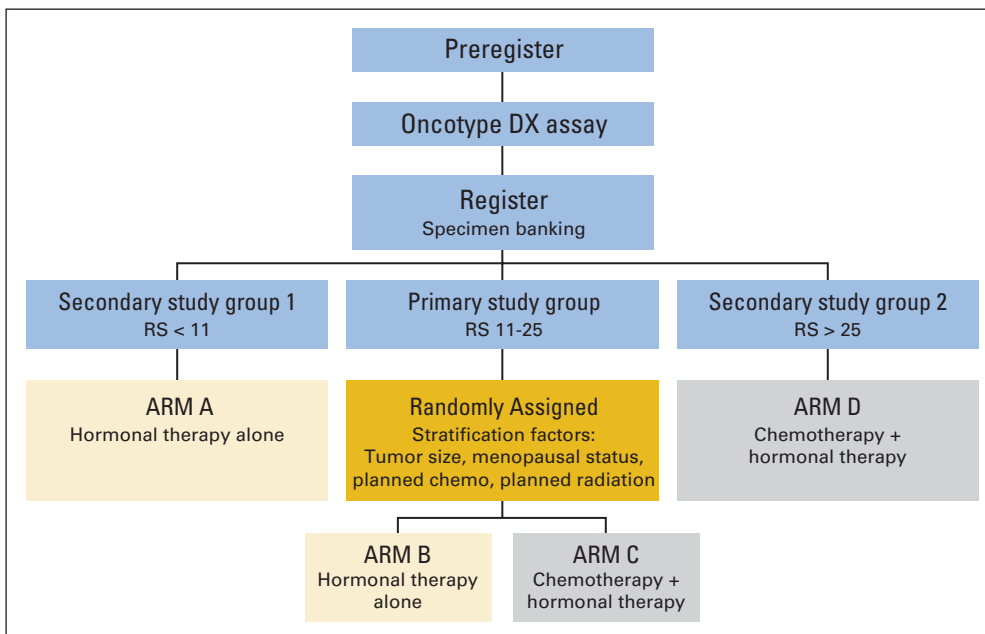
The Oncotype Dx assay has been ordered for more than 40,000 patients and by approximately 6,000 different physicians since it became commercially available in January, 2005 (personal communication, S. Shak, December 2007). In the postmarketing experience, including the first 20,050 tumors evaluated in the Genomic Health laboratory, the RS was low in 48%, intermediate in 37%, and high in 15% of patients; therefore, the distribution of low scores was comparable to the original validation studies, but the proportion with high scores was less, likely reflecting an appropriate degree of selection bias as clinicians began using the assay in clinical practice.

There have been three studies reported to date evaluating the impact of the 21-gene assay in clinical decision making that have shown similar findings.<sup>41-43</sup> (Table 2) First, as indicated by the postmarketing experience, fewer cases had a high RS because clinicians tend to order the assay for patients who have low- or intermediate-risk clinicopathologic features rather than high-risk features. Second, despite selection bias inherent in use of the assay, the results of the test altered treatment recommendation in approximately 25% of patients, usually resulting in “treatment sparing” (a change in recommendation from chemohormonal therapy to hormonal therapy alone) and less often in “treatment selection” (a change in recommendation from hormonal therapy to chemohormonal therapy). Although the cost of the test is high (\$3,500), an economic analysis suggested that that appropriate use of the test offered potential for cost savings.<sup>44</sup>

**Table 3.** Scientific Rationale for Selecting Oncotype Dx Assay for TAILORx

Rationale	Study
Validation study demonstrates that RS is a prognostic factor for risk of distant recurrence in tamoxifen treated patients that outperforms clinical criteria in multivariate model	Paik et al <sup>12</sup>
External population-based validation study demonstrating that RS is predictive of breast cancer death	Habel et al <sup>13</sup>
High RS is predictive of chemotherapy benefit in tamoxifen-treated patients	Paik et al <sup>34</sup>
RS correlates with risk of local recurrence	Mamounas et al <sup>51</sup>
RS predicts outcome more accurately than Adjuvant! in patients with negative nodes treated with tamoxifen	Bryant <sup>27</sup>
Concordance in predictive value of Oncotype Dx compared with other multigene markers	Fan et al <sup>49</sup>

NOTE. Oncotype Dx provided by Genomic Health, Redwood City, CA.  
Abbreviations: TAILORx, Trial Assigning Individualized Options for Treatment (Rx); RS, recurrence score.



**Fig 5.** Schema showing Trial Assigning Individualized Options for Treatment (TAILORx) design. RS, recurrence score.

#### RATIONALE FOR AND DESIGN OF TAILORX

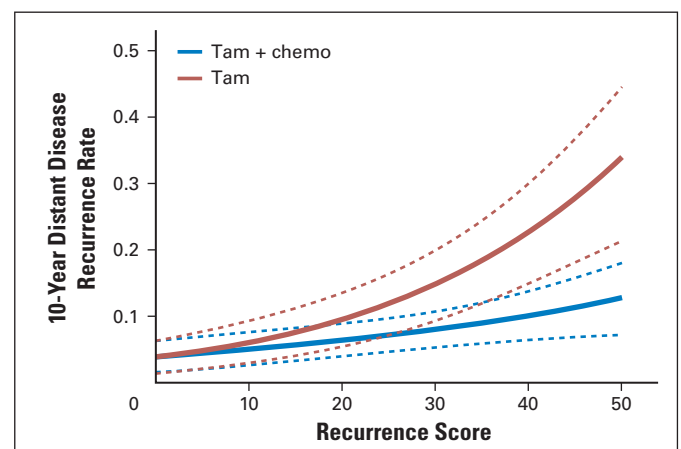
To address the challenge of integrating molecular diagnostic testing into clinical practice, the US National Cancer Institute initiated the Program for the Assessment of Clinical Cancer Tests (PACCT; <http://www.cancerdiagnosis.nci.nih.gov/assessment/index.htm>). This effort has led to publication of the REMARK guidelines (Reporting Recommendations for Tumor Marker Prognostic Studies),<sup>45</sup> and to TAILORx (<http://www.cancer.gov/clinicaltrials/digestpage/TAILORx>), which was developed by the North American Breast Cancer Intergroup. The key questions that the Intergroup grappled with in designing TAILORx included the population to be studied, the molecular marker to be evaluated, and a trial design that would be both informative and practical.<sup>46</sup>

With regard to the patient population, it became obvious that hormone receptor–positive, lymph node–negative breast cancer was an appropriate group to target because it is common (124,000 cases in the United States in 2006), accounting for approximately 50% of all breast cancer cases diagnosed each year, and nearly 9% of all new cases of cancer.<sup>47</sup> Chemotherapy is typically recommended if the residual risk of recurrence exceeds approximately 5% to 10% despite adjuvant hormonal therapy.<sup>48</sup> Although adding chemotherapy reduces the risk of recurrence on average by approximately 25% to 30%, the absolute benefit for an individual patient is small, ranging from 1% to 5%.<sup>24</sup> The vast majority of patients with ER-positive breast cancer are therefore overtreated with chemotherapy, considering that most would have been cured with hormonal therapy alone. Application of a molecular marker into the clinical decision-making process might offer the opportunity to spare the majority of patients who are overtreated with chemotherapy and who may be adequately treated with hormonal therapy alone.

With regard to the molecular marker selected, the Intergroup selected the 21-gene assay for the reasons summarized in Table 3. Key considerations included the fact that the assay was specifically devel-

oped for and validated in patients with hormone receptor–positive, lymph node–negative disease, was prognostic for both distant and local recurrence, and was predictive for treatment benefit for hormonal therapy (if the RS is low) and chemotherapy benefit (if the RS is high). A recent study suggesting concordance between the 21-gene assay and other multigene markers provides additional evidence supporting this choice,<sup>49</sup> although some have pointed out technical flaws in the comparative analysis.<sup>50</sup>

With regard to the trial design, the Intergroup concluded that it was important to design the trial in a manner that integrated what was already known about the assay into clinical practice, addressed a clinically important question, had a practical design, and provided the opportunity to evaluate other markers in the future. Because it was clear that low RS was associated with a low recurrence rate with



**Fig 6.** Linear fit of the likelihood of distant recurrence as a continuous function of recurrence score for the tamoxifen alone (Tam) and with chemotherapy (Tam + chemo) treatment groups in the B20 trial. Reprinted with permission from Paik S, Tang G, Shak S, et al: *J Clin Oncol* 24:3726-3734, 2006.<sup>31</sup>

**Table 4.** RS and Chemotherapy Response in Trial B-20 (N = 651)

RS	Patients		10-Year DRFS (%)		Recurrence by Addition of Chemotherapy		P	10-Year DFS (%)		Recurrence by Addition of Chemotherapy		P
	No.	%	Tam	Tam + Chemo	HR	95% CI		Tam	Tam + Chemo	HR	95% CI	
< 11	177	27	98	95	1.788	0.360 to 8.868	.471	77	85	0.605	0.317 to 1.153	.124
11-25	279	43	95	94	0.755	0.313 to 1.824	.531	81	76	1.106	0.671 to 1.823	.691
> 25	195	30	63	88	0.285	0.148 to 0.551	< .0001	53	75	0.446	0.270 to 0.738	.0012

NOTE. RS cutoffs as used in the TAILORx (Trial Assigning Individualized Options for Treatment [Rx]) trial. Chemotherapy included cyclophosphamide, methotrexate, fluorouracil, or methotrexate/fluorouracil.

Abbreviations: RS, recurrence score; DRFS, distant relapse-free survival; DFS, disease-free survival; Tam, tamoxifen; HR, hazard ratio.

hormonal therapy alone, and that high RS predicted substantial benefit from chemotherapy, there seemed to be sufficient level and evidence to make definitive treatment recommendations for patients who fell into these groups. For patients with a midrange RS and who met standard clinicopathologic criteria for recommending adjuvant chemotherapy, however, there remained uncertainty about the best treatment option. These considerations led to the TAILORx trial design shown in Figure 5. Treatment is assigned for patients who have a low RS (hormonal therapy alone) or high RS (chemohormonal therapy), and those who have a midrange RS are randomly assigned to chemohormonal therapy (the standard treatment arm) or hormonal therapy alone (the experimental arm). The trial utilizes a noninferiority design for the midrange group, and is adequately powered to detect a 3% or greater difference between the randomized arms. At the time of registration and treatment assignment, patients are asked to provide blood samples for banking of plasma and peripheral-blood mononuclear cells. The trial is open at over 900 sites in the United States and Canada, and several international sites in Ireland, South America, and Israel, and is projected to complete its accrual goal in 2009.

The RS ranges used in TAILORx are different from those originally defined as low- (< 18), intermediate- (18-30), and high- ( $\geq$  31) risk. The range was adjusted to minimize the potential for undertreatment in both the high-risk group and the randomized group. When the NSABP B-20 data were analyzed using the RS ranges used in TAILORx, the treatment effect of chemotherapy was similar to the original analysis, and the risk of recurrence was 5% or less with tamoxifen alone in the low and midrange RS groups (Table 4). Although a trend favoring the addition of chemotherapy becomes evident at an RS of approximately 11 when the risk of relapse is analyzed in a linear fashion, the 95% CIs completely overlap in the 11 to 25 RS range (Fig 6). An RS of 11 is associated with a risk of both local and distant relapse of approximately 10%, a threshold that has been typically used for recommending adjuvant chemotherapy.

Selection bias in the clinical application of the assay is evidenced by examining the distribution of RS ranges in the postmarketing

experience and in TAILORx. In the postmarketing experience involving 20,500 tests ordered commercially, the RS distribution using the TAILORx definitions was 14% low, 62% midrange, and 24% high, and is similar to the RS distribution observed in the B-20 trial shown in Table 4.<sup>39</sup> As of September 19, 2007, of the 1,974 patients registered on TAILORx, 13% had a low-risk, 70% midrange, and 17% high-risk TAILORx RS, which is quite similar to the postmarketing experience; approximately 60% of patients enrolled have a tumor between 1 and 2 cm in size, and 60% have an intermediate grade.

## CONCLUSION

There is a substantial body of evidence supporting the clinical utility of the 21-gene assay in patients with hormone receptor-positive, axillary lymph node-negative disease. By integrating this molecular diagnostic test into the clinical decision-making process in TAILORx, patients and clinicians will be able to make more informed decisions regarding the most appropriate treatment options for a subset of patients for whom the test provides a clear treatment path. In addition, researchers may be able to refine the utility of the assay, and evaluate other genomic markers and other technologies as they are developed.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Conception and design:** Joseph A. Sparano, Soonmyung Paik  
**Collection and assembly of data:** Joseph A. Sparano, Soonmyung Paik  
**Manuscript writing:** Joseph A. Sparano, Soonmyung Paik  
**Final approval of manuscript:** Joseph A. Sparano, Soonmyung Paik

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