O R I G I N A L   R E P O R T

Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor–Positive Breast Cancer

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A B S T R A C T

Purpose
The 21-gene recurrence score (RS) assay quantifies the likelihood of distant recurrence in women with estrogen receptor–positive, lymph node–negative breast cancer treated with adjuvant tamoxifen. The relationship between the RS and chemotherapy benefit is not known.

Methods
The RS was measured in tumors from the tamoxifen-treated and tamoxifen plus chemotherapy–treated patients in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B20 trial. Cox proportional hazards models were utilized to test for interaction between chemotherapy treatment and the RS.

Results
A total of 651 patients were assessable (227 randomly assigned to tamoxifen and 424 randomly assigned to tamoxifen plus chemotherapy). The test for interaction between chemotherapy treatment and RS was statistically significant ($P = .038$). Patients with high-RS (≥ 31) tumors (ie, high risk of recurrence) had a large benefit from chemotherapy (relative risk, 0.26; 95% CI, 0.13 to 0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; SE, 8.0%). Patients with low-RS (< 18) tumors derived minimal, if any, benefit from chemotherapy treatment (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%). Patients with intermediate-RS tumors did not appear to have a large benefit, but the uncertainty in the estimate can not exclude a clinically important benefit.

Conclusion
The RS assay not only quantifies the likelihood of breast cancer recurrence in women with node-negative, estrogen receptor–positive breast cancer, but also predicts the magnitude of chemotherapy benefit.

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INTRODUCTION

The use of adjuvant chemotherapy in the treatment of early-stage node-negative breast cancer has increased greatly in the last decade in the United States.[1] Ideally, the decision to use chemotherapy in addition to hormonal therapy in the treatment of axillary node–negative and estrogen receptor (ER)–positive breast cancer should be based on not only baseline risk (prognostic information) but also prediction of degree of benefit from chemotherapy.[2,3] Current treatment guidelines in the United States and Europe recommend consideration of chemotherapy for the vast majority of patients.[4-7]

A number of biologic and clinical clues have suggested that not all patients derive the same degree of benefit from chemotherapy. An overview of randomized trials suggests that younger women may benefit more from chemotherapy than older women.[8,9] Diagnostic tests that predict clinical benefit from chemotherapy are not yet available for routine clinical use.

Gene expression analysis in individual tumors is a promising approach for defining responsiveness to chemotherapy.[10] Several small studies have been performed in breast cancer patients treated with neoadjuvant chemotherapy before surgery, and gene expression profiles have been associated with the likelihood of pathologic complete response.[11-13]

Taking advantage of National Surgical Adjuvant Breast and Bowel Project (NSABP) clinical trials archived paraffin block tissue bank, we have

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developed and validated a 21-gene assay that is now offered as a commercial reference laboratory test (Oncotype DX, Genomic Health Inc, Redwood City, CA).[14,15] The 21-gene panel includes genes involved in tumor cell proliferation and hormonal response, characteristics that have been reported to be associated with chemotherapy response in general. We explored the interaction between Oncotype DX assay and chemotherapy benefit using tissue blocks collected from NSABP trial B20 which tested the worth of adding cyclophosphamide, methotrexate, and fluorouracil (CMF) or methotrexate and fluorouracil (MF) chemotherapy to 5 years of tamoxifen in the treatment of node-negative, ER-positive patients.[16] We sought to determine whether the prespecified 21-gene reverse-transcriptase polymerase chain reaction (RT-PCR) assay predicts the benefit of chemotherapy in the NSABP B20 patients.

METHODS

Patients

NSABP Protocol B20, "A Clinical Trial to Determine the Worth of Chemotherapy and Tamoxifen Over Tamoxifen Alone in the Management of Patients with Primary Invasive Breast Cancer, Negative Axillary Nodes and Estrogen-Receptor-Positive Tumors," enrolled 2,363 patients between October 17,1988, and March 5, 1993, who were randomly assigned to tamoxifen versus tamoxifen plus either CMF or MF.[16] This study was approved by the Essex Institutional Review Board (IRB; Lebanon, NJ), the Allegheny General Hospital IRB (Pittsburgh, PA), and the University of Pittsburgh IRB (Pittsburgh, PA).

Sample Preparation, Genes, Recurrence Score Algorithm

Gene expression in fixed paraffin embedded tumor tissue was performed by Genomic Health Inc, using the previously described Oncotype DX assay.[14,15] After RNA extraction and DNase I treatment, total RNA content was measured, and the absence of DNA contamination was verified. Gene-specific reverse transcription was performed followed by quantitative TaqMan RT-PCR reactions in 384 well plates using Applied Biosystems (Foster City, CA) PRISM 7900HT instruments.

Expression of each gene was measured in triplicate, and normalized relative to a set of five reference genes (beta-actin [ACTB], GAPDH, GUS, RPLPO, and TFRC). Reference-normalized expression measurements range from 0 to 15; a one-unit increase reflects approximately a two-fold increase in RNA.

The recurrence score (RS) is calculated on a scale from 0 to 100 and is derived from the reference-normalized expression measurements for the 16 cancer-related genes (Ki67, STK15, Survivin or BIRC5, CCNB1 or cyclin B1, MYBL2, GRB7, HER2, ER, PGR, BCL2, SCUBE2, MMP11 or stromelysin 3, CTSL2 or cathepsin L2, GSTM1, CD68, and BAG1) and the five reference genes.

The cutoff points that were prespecified before the performance of the validation study of the RS,[15] which categorized patients into low-risk (RS < 18), intermediate-risk (RS ≥ 18 and < 31), and high-risk (RS ≥ 31) groups, were also prespecified in this study.

Study Design and End Points

Patients were eligible if a tumor block was available in the NSABP Tumor Bank. Exclusion criteria were insufficient tumor (< 5% of the overall tissue) as assessed by histopathology, insufficient RNA (< 0.5 μg), or weak RT-PCR signal (average cycle threshold for the reference genes > 35).

The objective of this study was to determine whether the RS predicts the magnitude of chemotherapy benefit. Because there was no significant difference between the two chemotherapy treatments in the overall study, we prespecified that the two chemotherapy arms would be combined for the primary analysis. Although the tamoxifen arm of B20 had been analyzed as a training set in the development of the Oncotype DX assay as previously reported,[15,17] a preliminary version of the RT-PCR assay (lacking stan-

dardized reagents, calibrators, and controls) had been used. Therefore, for this study, the tamoxifen arm was analyzed again using the commercial assay (Oncotype DX). The B20 samples from the chemotherapy-treated patients had not been analyzed previously.

The primary prespecified end point was freedom from distant recurrence. Contralateral disease, other second primary cancers, and deaths before distant recurrence were considered censoring events. Ipsilateral breast recurrence, local chest wall recurrence, and regional recurrences were not considered either as events or as censoring events.

ER and progesterone receptor (PR) were measured by the ligand-binding assay.[16] Tumor grade was determined centrally by two board-certified pathologists (T.G. and F.L.B.) from Stanford University Medical Center (Stanford, CA) and University of California, San Francisco, School of Medicine (San Francisco, CA) using the Elston modification of the Bloom-Richardson grading criteria.[18] In addition, histologic grade reported by the site pathologist was analyzed.

Statistical Analysis

Cox proportional hazards models were utilized to examine the interaction between chemotherapy treatment and RS as a continuous variable. The likelihood ratio test for interaction compared the reduced model, which excluded the RS by treatment interaction, with the competing full model, which included the RS by treatment interaction. A P value less than .05 for the likelihood ratio test was considered significant. Analysis was also performed using the predefined RS risk categories (low, intermediate, and high risk). Secondary analyses were performed to examine clinical variables as well as the individual genes and gene group scores (eg, proliferation gene group score) that define the RS for their main effects and for their interaction with treatment.

RESULTS

Patient Demographics

There were 2,299 clinically eligible patients. Blocks containing sufficient invasive breast cancer were available for 670 patients. In the remaining patients, the blocks were either never obtained by NSABP or were exhausted from use in prior studies. RT-PCR was successful in more than 97% of the blocks that were analyzed, and gene expression results were obtained in 651 patients, 227 of 770 tamoxifen-treated and 424 of 1,529 chemotherapy-treated clinically eligible patients. Distributions of patient age, tumor size, tumor grade, and hormone status in the 651 patients assessable for this study were similar to those in all 2,299 clinically eligible NSABP B20 patients (Table A1, online-only appendix).

Among the 651 assessable patients with RT-PCR assay data, the Kaplan-Meier estimate for the proportion of patients without distant recurrence at 10 years was 92.2% for patients treated with tamoxifen plus chemotherapy and 87.8% for those treated with tamoxifen alone. Kaplan-Meier estimates for the proportion of patients without any relapse (locoregional or distant) at 10 years were 90.1% and 83.5%, respectively. Kaplan-Meier estimates for overall survival at 10 years were 89.5% and 86.4%, respectively.

Correlation Between RS and Standard Prognostic Factors

There were 353 patients (54.2%) in the low-risk group (RS < 18), 134 patients (20.6%) in the intermediate-risk group (RS 18 to 30), and 164 patients (25.2%) in the high-risk group (RS ≥ 31).

Figure 1 shows the relationship between the RS and other prognostic factors, including patient age at the time of diagnosis, tumor size, hormone-receptor status (measured by biochemical assay), and histologic grade performed by two central pathologists as well as the

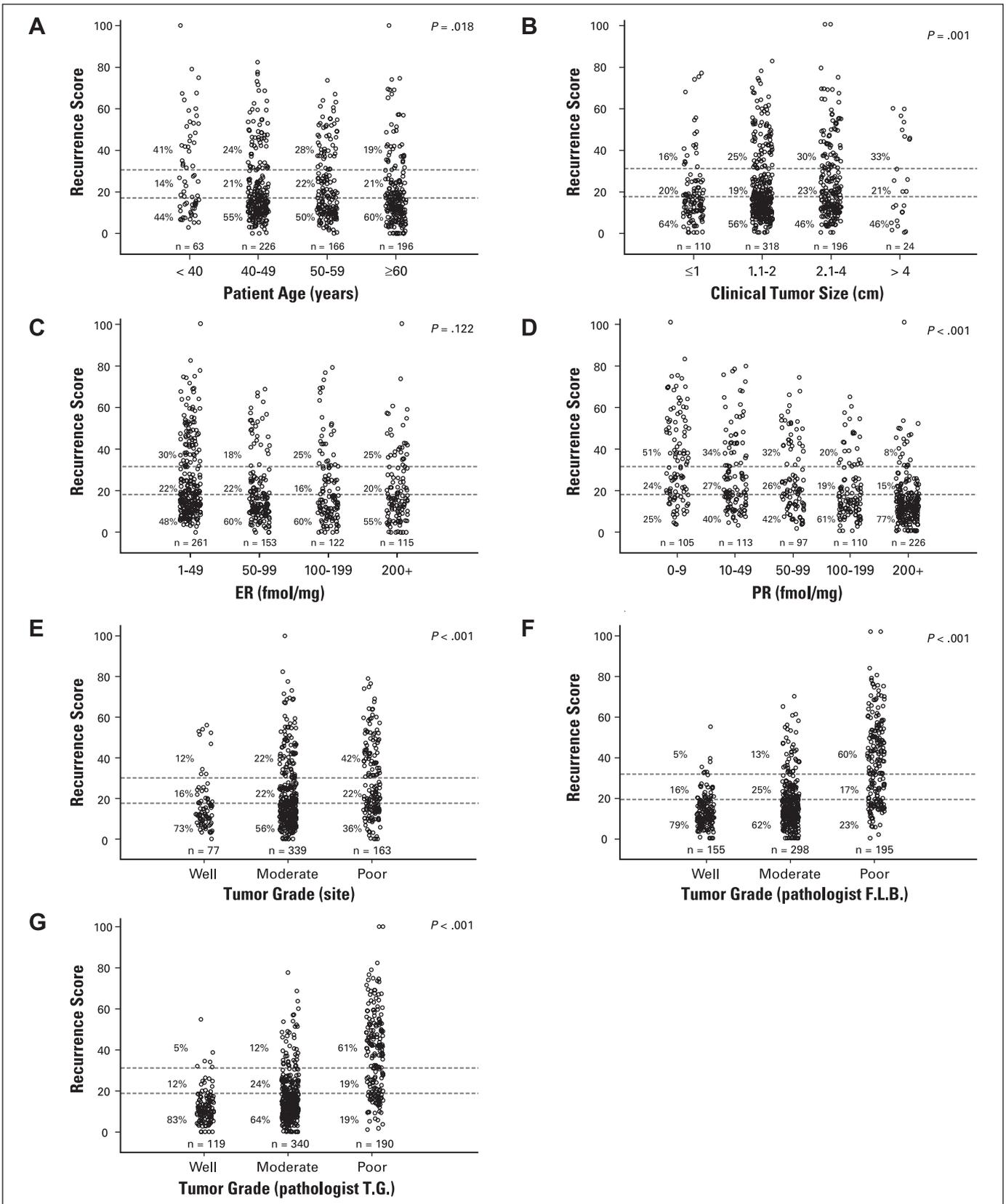**Fig 1.** Distributions of recurrence score (RS) and standard prognostic factors. (A) RS *v* age; (B) RS *v* clinical tumor size; (C) RS *v* estrogen receptor (ER) by ligand binding; (D) RS *v* progesterone receptor (PR) by ligand binding; (E) RS by tumor grade (National Surgical Adjuvant Breast and Bowel Project site pathologists); (F) RS by tumor grade (F.L.B.); (G) RS by tumor grade (T.G.).

grade reported in the site's pathology reports. There was a modest concordance between RS and patient age. Although RS was associated with PR by ligand-binding and poor histologic grade, a large numbers of cases were discordant. For example, there were cases of tumors with low RSs that had low PR by ligand-binding or poor tumor grade. In addition, there was only modest agreement in assessment of histologic grade among the three pathologists (Tables A2-A4, online-only appendix).

Effect of Chemotherapy for Patient Groups Defined by the RS

The Kaplan-Meier plots and estimates of the proportion of patients distant recurrence–free for each of the RS risk categories are shown in Figure 2 and Table 1, respectively. The magnitude of the chemotherapy benefit was greater for the high-risk patients (RS ≥ 31) than for the intermediate- (RS, 18 to 30) or low-risk patients (RS < 18). There was a large benefit of chemotherapy in the high-risk patients, whereas there was minimal, if any, benefit of chemotherapy in the low-risk patients. The 10-year Kaplan-Meier estimate for freedom from distant recurrence was improved from 60% to 88% by adding chemotherapy to tamoxifen in the high-risk group.

The relative and absolute benefits of chemotherapy for RS risk groups are shown in Figure 3. Although no demonstrable reduction in distant recurrence at 10 years for the predefined low risk category was evident (relative risk, 1.31; 95% CI, 0.46 to 3.78; increase of 1.1% in absolute risk), a large reduction in distant recurrence at 10 years was evident for the high-risk category (relative risk, 0.26; 95% CI, 0.13 to 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 to 1.59; 1.8% increase in absolute risk). Similar trends were observed for freedom from locoregional and/or distant recurrence and overall survival (Figs A1 and A2, online-only appendix).

Similar results were observed when chemotherapy benefit was analyzed in the CMF and MF groups separately (data not shown).

Relationship of the RS and Chemotherapy Treatment Benefit

To test the statistical strength of the relationship between the magnitude of chemotherapy benefit and RS, a formal test of statistical interaction between the RS as a continuous variable and chemotherapy treatment was performed. In a multivariate analysis

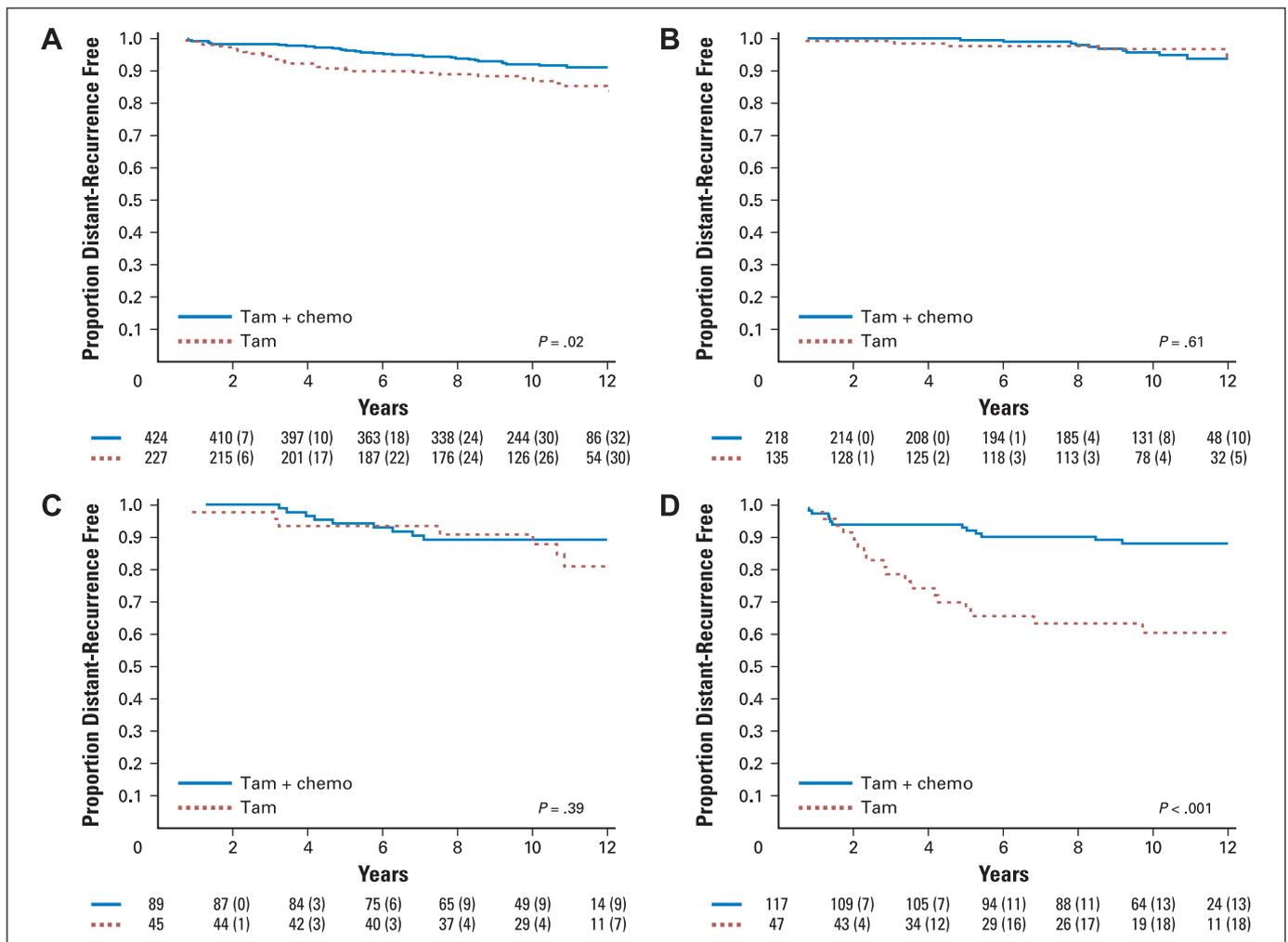**Fig 2.** Kaplan-Meier plots for distant recurrence comparing treatment with tamoxifen (Tam) alone versus treatment with tamoxifen plus chemotherapy (Tam + chemo). (A) All patients; (B) low risk (recurrence score [RS] < 18); (C) intermediate risk (RS 18-30); (D) high risk (RS ≥ 31). The number of patients at risk and the number of distant recurrences (in parentheses) are provided below each part of the figure.

Table 1. Kaplan-Meier Estimates of the Proportion of Patients Free of Distant Recurrence at 10 Years for Tamoxifen-Treated Patients and Tamoxifen Plus Chemotherapy-Treated Patients

		Tamoxifen			Tamoxifen Plus Chemotherapy		
Group	No. of Patients	10-Year DRF (%)	95% CI	No. of Patients	10-Year DRF (%)	95% CI	No. of Patients
All patients	651	87.8	83.3% to 92.3%	227	92.2	89.4% to 94.9%	424
Low risk (RS < 18)	353	96.8	93.7% to 99.9%	135	95.6	92.7% to 98.6%	218
Intermediate risk (RS 18-30)	134	90.9	82.5% to 99.4%	45	89.1	82.4% to 95.9%	89
High risk (RS ≥ 31)	164	60.5	46.2% to 74.8%	47	88.1	82.0% to 94.2%	117

NOTE. Results are given for all patients and for the pre-specified Recurrence Score risk categories.
Abbreviations: DRF, distant recurrence free; RS, recurrence score.

of Cox models containing chemotherapy treatment and RS as a continuous variable without and with the addition of a term for interaction between chemotherapy treatment and RS, the likelihood ratio test for interaction between chemotherapy treatment and RS was significant ($P = .038$).

In addition, individual multivariate models for the interaction between RS and chemotherapy treatment that were adjusted for other variables (including patient age, tumor size, ER, PR, tumor grade by each pathologist, including site, G.T., and F.L.B.) demonstrated persistence of the strength of the interaction between RS and chemotherapy treatment ($P = .035$ to $.068$).

To explore the degree of benefit from chemotherapy in relationship to RS as a continuous function, the likelihood of distant recurrence was fit as a linear function of the RS for both the tamoxifen alone and tamoxifen plus chemotherapy arms (Fig 4). A linear fit was used because the tests for nonlinearity of RS in Cox proportional hazards models were not significant in this study ($P = .32$ and $.73$ for the tamoxifen and tamoxifen plus chemotherapy arms, respectively).[19] The magnitude of chemotherapy benefit appeared to increase continuously as the RS increased. A clear cutoff point for RS, below which there is no demonstrable benefit from chemotherapy, cannot be accurately defined.

Gene Expression and Chemotherapy Benefit: Clinical Variables, Individual Genes, and Gene Groups

The interaction of chemotherapy treatment with the clinical variables and with all the individual genes and gene group scores are shown in Table 2.

For the 651 assessable patients, there was no significant interaction between the clinical variables and chemotherapy treatment. Hazard ratios for the interaction of age, hormone receptors, and tumor grade with chemotherapy treatment were, however, in the anticipated directions.
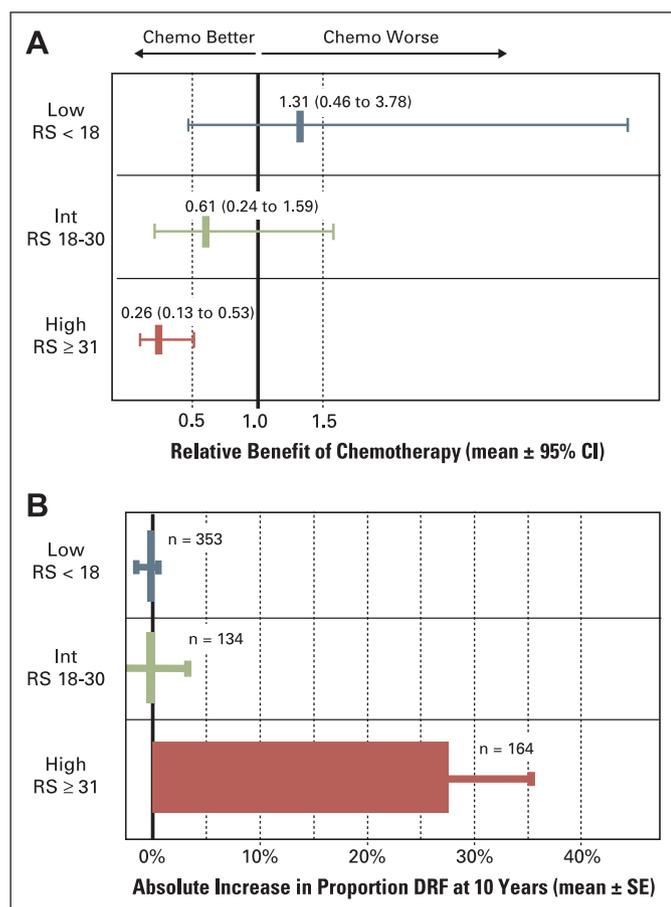

Fig 3. Relative and absolute risks of chemotherapy (chemo) benefit as a function of recurrence score (RS) risk category. Int, intermediate; DRF, distant recurrence free.
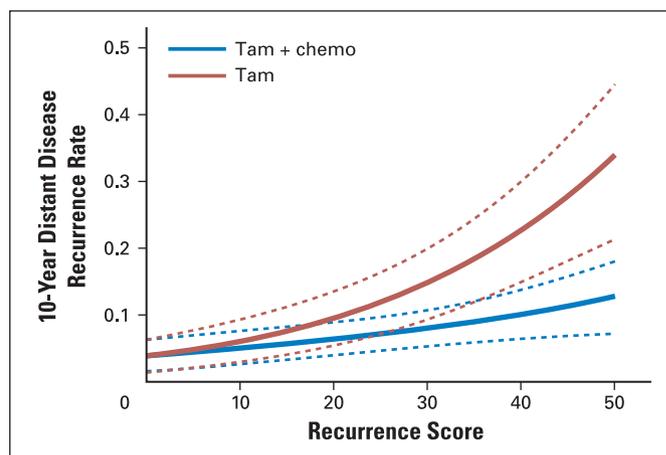

Fig 4. Linear fit of the likelihood of distant recurrence as a continuous function of recurrence score for the tamoxifen alone (TAM) and tamoxifen plus chemotherapy (TAM + chemo) treatment groups.

Table 2. Likelihood Ratio Tests of the Interaction of Chemotherapy Treatment With the Clinical Variables and the Gene Expression Variables

Variable	Assessable B20 Patients (n = 651)				All B20 Patients (N = 2,299)			
	HR	Lower 95%	Upper 95%	P	HR	Lower 95%	Upper 95%	P
Clinical variables								
Age ≥ 50 years*	2.02	0.75	5.47	.162	1.78	1.06	2.97	.029
Tumor size > 2 cm†	1.34	0.49	3.68	.569	0.76	0.45	1.27	.293
Quantitative ER ≥ 50‡	1.96	0.73	5.30	.183	1.54	0.92	2.57	.099
Quantitative PR ≥ 50‡	1.87	0.70	4.97	.214	0.76	0.45	1.27	.289
Grade site§								
Poor	0.27	0.02	3.01	.284	0.31	0.09	1.04	.057
Moderate	0.60	0.06	6.42	.672	0.51	0.15	1.70	.273
Grade, pathologist A								
Poor	0.73	0.19	2.89	.657	—	—	—	—
Moderate	1.04	0.23	4.58	.963	—	—	—	—
Grade, pathologist B					—	—	—	—
Poor	0.32	0.06	1.77	.192	—	—	—	—
Moderate	0.36	0.06	2.03	.244	—	—	—	—
Gene expression variables‖					—	—	—	—
Recurrence score¶	0.32	0.11	0.94	.038	—	—	—	—
Proliferation gene group-TH**	0.33	0.11	0.94	.039	—	—	—	—
MYBL2	0.67	0.45	1.00	.050	—	—	—	—
Invasion gene group**	0.52	0.27	1.02	.056	—	—	—	—
SCUBE2	1.25	0.99	1.58	.063	—	—	—	—
ER gene group**	1.32	0.95	1.84	.094	—	—	—	—
GSTM1	1.33	0.94	1.88	.102	—	—	—	—
CTSL2	0.67	0.41	1.09	.108	—	—	—	—
Proliferation gene group**	0.64	0.35	1.15	.134	—	—	—	—
Ki-67	0.66	0.36	1.19	.167	—	—	—	—
PR	1.16	0.93	1.46	.190	—	—	—	—
BAG1	1.54	0.75	3.16	.240	—	—	—	—
SURV	0.79	0.53	1.18	.243	—	—	—	—
CCNB1	0.67	0.33	1.36	.269	—	—	—	—
ER	1.13	0.86	1.48	.393	—	—	—	—
STMY3	0.86	0.57	1.29	.458	—	—	—	—
Bcl2	0.87	0.54	1.39	.564	—	—	—	—
HER2 gene group-TH**	0.89	0.57	1.39	.610	—	—	—	—
STK15	0.86	0.47	1.56	.615	—	—	—	—
CD68	1.11	0.54	2.26	.779	—	—	—	—
GRB7	0.98	0.67	1.42	.904	—	—	—	—
HER2 gene group**	0.98	0.67	1.43	.910	—	—	—	—
HER2	0.98	0.67	1.43	.925	—	—	—	—

Abbreviations: HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; TH, with threshold.

*Age at surgery categorized as a binary factor: 0 = age at surgery < 50 years, 1 = age at surgery ≥ 50 years.

†Clinical tumor size categorized as a binary factor: 0 = size ≤ 2 cm, 1 = size > 2 cm.

‡Hormone receptors by ligand binding were categorized as binary variables: quantitative ER ≥ 50 fmol/mg was compared with ER < 50 fmol/mg, and quantitative PR ≥ 50 fmol/mg was compared with PR < 50 fmol/mg.

§Grade was categorized as binary variables: poorly differentiated was compared with well differentiated, and moderately differentiated was compared with well differentiated.

‖Individual genes and gene groups, unless otherwise specified, were used as a continuous variable, with HR for the interaction relative to a one unit increment in gene expression. HR < 1.0 indicates that higher expression is associated with greater chemotherapy benefit; HR > 1.0 indicates that higher expression is associated with lesser chemotherapy benefit.

¶Recurrence score used as a continuous variable, with HR for the interaction relative to an increment of 50 units.

**Proliferation gene group-TH and HER2 gene group-TH are treated as transformed variables using the calculation prespecified in the formula for the recurrence score.[15] The proliferation gene group = (SURV + Ki-67 + MYBL2 + Cyclin B1 + STK15)/5; the proliferation gene group-TH = (SURV + Ki-67 + MYBL2 + Cyclin B1 + STK15)/5 if ≥ 6.5 and 6.5 if < 6.5. The HER2 gene group = 0.9 × GRB7 + 0.1 × HER2; the HER2 gene group-TH = 0.9 × GRB7 + 0.1 × HER2 if ≥ 8.0 and 8.0 if < 8.0. The invasion gene group = (CTSL2 + STMY3)/2. The ER gene group = (0.8 × ER + 1.2 × PR + Bcl2 + SCUBE2)/4.

There was a trend for higher expression by RT-PCR of each of the five proliferation genes to be associated with chemotherapy benefit. There was a trend for lower expression of PR, ER, and the ER gene group to be associated with chemotherapy benefit. No interaction was evident between either HER2 or GRB7 and chemotherapy treatment.

For the clinical variables, we also examined the interaction with chemotherapy treatment in the entire NSABP B20 cohort as well. As reported previously,[8] the interaction between chemotherapy treatment and patient age reached statistical significance ($P < .05$) when the entire B20 cohort was examined. However, the degree of interaction of chemotherapy treatment and gene expression is stronger than that for the clinical variables, suggesting that the RS is the strongest predictor of chemotherapy benefit.

DISCUSSION

Patients with node-negative, ER-positive breast cancer in the NSABP B20 study did not benefit equally from chemotherapy. The prespecified 21-gene RT-PCR assay predicted the magnitude of benefit of either CMF or MF chemotherapy. The likelihood ratio test for interaction between chemotherapy treatment and RS was statistically significant ($P = .038$). Patients with tumors who had high RSs (≥ 31) experienced a large chemotherapy benefit (relative risk, 0.26; 95% CI, 0.13 to 0.53), with a mean absolute decrease in distant recurrence rate at 10 years of 27.6% (SE, 8.0%). Patients with tumors that had low RSs (RS < 18) derived minimal, if any, benefit from chemotherapy treatment (relative risk, 1.31; 95% CI, 0.46 to 3.78), with a mean absolute decrease in distant recurrence rate at 10 years of -1.1% (SE, 2.2%). Patients with tumors that had intermediate RSs (RS, 18 to 30) did not appear to receive a substantial benefit, but the uncertainty in the estimate (relative risk, 0.61; 95% CI, 0.24 to 1.59) can not exclude a clinically important benefit from chemotherapy treatment.

It should be noted that samples from the NSABP B20 tamoxifen-treated patients were used in one of the three studies used to select the 21 genes and to design the RS algorithm.[15,17] However, samples from the NSABP B20 tamoxifen plus chemotherapy–treated patients were not previously analyzed or used in the Oncotype DX development. Moreover, results from analysis of the NSABP B14 tamoxifen-treated patients (also not used in the development of the RS) were very similar to the results from analysis of the NSABP B20 tamoxifen-treated patients.

Few previous studies have examined biomarkers in patient cohorts from randomized trials comparing chemotherapy versus no chemotherapy. Studies that have examined the relationship between the response of tumors to chemotherapy and factors such as histologic grade and DNA ploidy have yielded inconsistent results.[20-25] However, tumors with characteristics associated with greater aggressiveness (eg, poor histologic grade, high levels of uPA/PA-1, ER negativity, high proliferative index) tended to respond better to chemotherapy.[26-29] In addition, there were trends for statistical interaction between clinical variables and chemotherapy benefit when all the NSABP B20 patients were examined (Table 2).[8] The 21-gene RT-PCR assay brings a high degree of precision and standardization to the quantification of important biologic characteristics of individual breast cancers. The RS calculation integrates the contribution of proliferation related genes and hormone receptor pathway genes. The data from this study indicates that greater chemotherapy benefit is observed in patients whose tumors have high RSs.

The clinical implications of these results for patients with low or relatively high RSs are relatively clear. For many women with low RSs, the anticipated benefit of adding chemotherapy to hormonal therapy may not exceed the risks. For many women with high RSs, the antici-

pated benefit of adding chemotherapy appears to be very favorable when compared with the risks. However, for women with intermediate RSs, it is uncertain that the benefits of chemotherapy exceed the risks. Additional study of the benefits and risks of chemotherapy in this middle range of patients is needed. The National Cancer Institute (NCI; National Institutes of Health, Bethesda, MD) Program for the Assessment of Clinical Cancer Tests (PACCT) Strategy Group, in collaboration with US Breast Intergroup, including NSABP, is considering a study that will further refine our ability to select patients for chemotherapy with RSs in the middle range.[30] However the results of the planned study will be available only after 2010, and until such time the results of Oncotype DX assay will have to be considered in the context of other clinical parameters in the selection of candidates for chemotherapy.

These data have implications with respect to cancer pathogenesis and metastasis. Adjuvant chemotherapy targets micrometastatic cells present after removal of primary tumor. Success in predicting chemotherapy benefit using analysis of the primary tumor implies that primary tumor and micromestastatic cells share molecular characteristics. Recent studies indicate that, overall, tumor molecular signatures tend to be preserved across different stages of tumor progression.[31,32] Our published studies with the 21-gene RS as well as other gene expression profiling studies demonstrate that expression profiling of the primary tumor can predict distant disease recurrence.[15,31,33,34] However, eventual tissue tropism of the metastases or survival of tumor cells in specific organ sites might require differential expression of certain genes in only a subset of the original tumor population.[35,36] Animal model system results from Massague's group[35] suggest that although metastatic cells need to acquire a specific set of genes for organotropic metastasis, their basic driving force is still governed by poor-prognosis genes in the primary tumor. Our results suggest that poor prognosis genes also influence chemotherapy response.

The relevance of this study with CMF chemotherapy to other regimens is under investigation. In a study of 89 patients with locally advanced breast cancer, Gianni et al[13] found that the RS also positively correlated with the probability of a pathologic complete response to neoadjuvant treatment with an anthracycline/taxane regimen. Patients with tumors with low RSs (< 18) rarely had a pathologic complete response. This suggests that relationship between the RS and chemotherapy benefit is not regimen specific. Whether there are specific predictor genes for particular chemotherapeutic drugs or regimens is not yet established. The NSABP trial B40 has been designed to address this question.

In summary, the RS assay not only quantifies the likelihood of breast cancer recurrence in women with node-negative, ER-positive breast cancer, it also predicts the magnitude of chemotherapy benefit. The results of this and other studies strongly suggest that not all women with breast cancer benefit equally from chemotherapy.

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Acknowledgment

Appendix

Table A1. Baseline Patient Characteristics

Characteristic	Genomic Health Population (n = 651)		Remaining Population (n = 1,648)		All B20 Patients (n = 2,299)	
	No.	%	No.	%	No.	%
Age at enrollment,* years						
<40	63	10	180	11	243	11
40-49	226	35	572	35	798	35
50-59	166	25	478	29	644	28
≥ 60	196	30	418	25	614	27
Clinical tumor size,* cm						
0-1.0	110	17	341	21	451	20
1.1-2.0	318	49	801	50	1,119	49
2.1-4.0	196	30	423	26	619	27
> 4.0	24	4	55	3	79	4
ER by ligand binding (fmol/mg)*						
10-49	261	40	762	46	1,023	44
50-99	153	23	347	21	500	22
100-199	122	19	292	18	410	18
≥ 200	115	18	251	15	366	16
PR by ligand binding (fmol/mg)*						
0-9	105	16	305	18	410	18
10-49	113	17	317	19	430	18
50-99	97	15	248	15	345	15
100-199	110	17	273	17	383	17
≥ 200	226	35	505	31	731	32
NSABP Site tumor grade†						
Well-differentiated	77	13	249	18	326	16
Moderately differentiated	339	59	768	56	1,107	57
Poorly differentiated	163	28	357	26	520	27

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; NSABP, National Surgical Adjuvant Breast and Bowel Project.
*χ^2 $P > .05$.
†χ^2 significant at $P = .03$.

Table A2. Pairwise Comparison of Assessments of Tumor Grade by Two Central Pathologists

	Pathologist (T.G.)			
Pathologist (F.L.B.)	Well	Moderate	Poor	Total
Well	91	59	4	154
Moderate	28	237	33	298
Poor	0	42	153	195
Total	119	338	190	647

NOTE. Concordance = 71%; kappa = 0.59.

Table A3. Pairwise Comparison of Assessments of Tumor Grade by Central Pathologist (F.L.B.) and the NSABP Study Site Pathologist

	Site Pathologist			
Pathologist (F.L.B.)	Well	Moderate	Poor	Total
Well	37	69	29	135
Moderate	33	172	59	264
Poor	7	97	73	177
Total	77	338	161	576

NOTE. Concordance = 49%; kappa = 0.17.
Abbreviation: NSABP, National Surgical Adjuvant Breast and Bowel Project.

Table A4. Pairwise Comparison of Assessments of Tumor Grade by Central Pathologist (T.G.) and the NSABP Study Site Pathologist

	Site Pathologist			
Pathologist (T.G.)	Well	Moderate	Poor	Total
Well	31	57	20	108
Moderate	37	195	67	299
Poor	9	86	75	170
Total	77	338	162	577

NOTE. Concordance = 52%; kappa = 0.19.
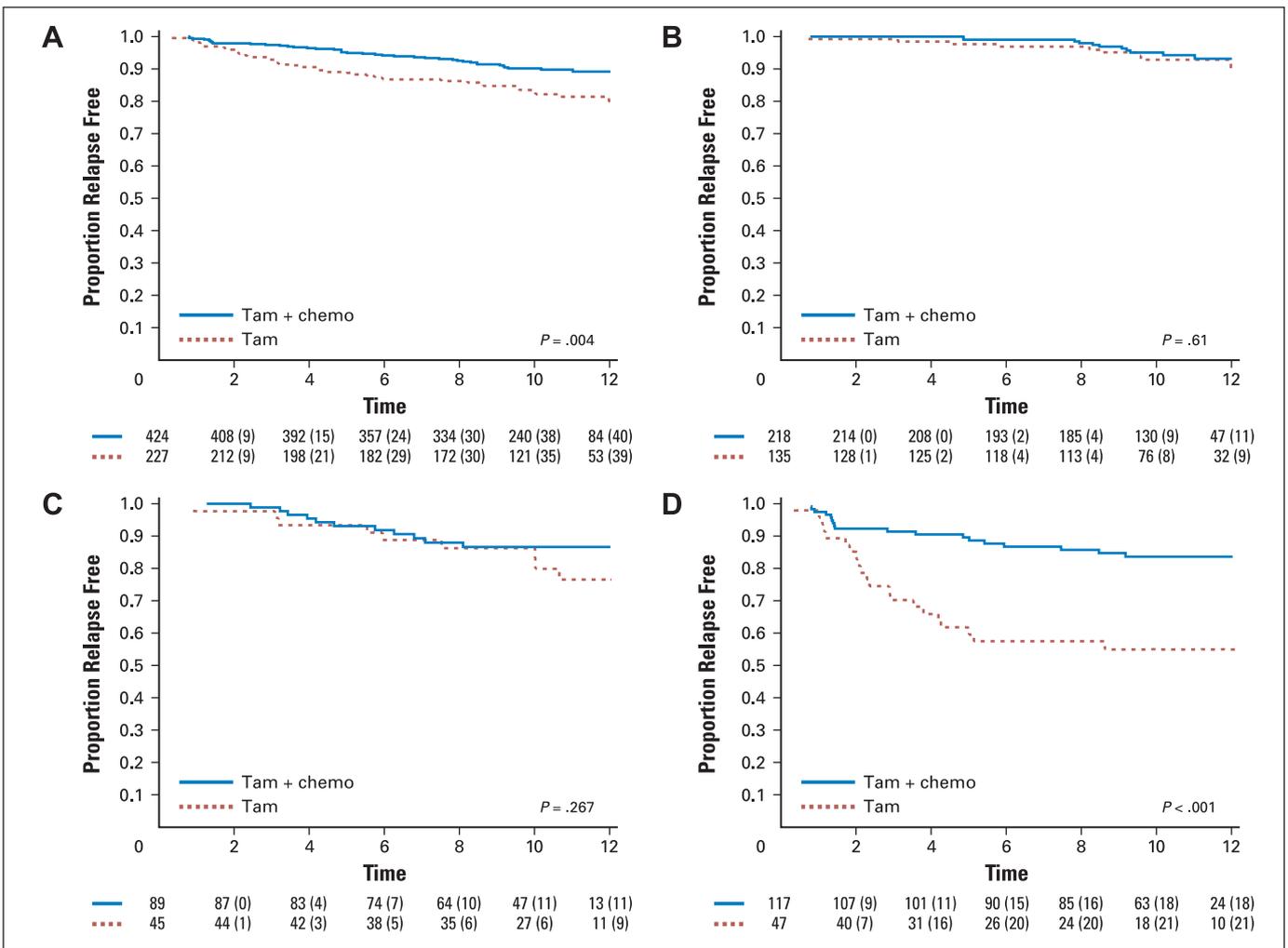Abbreviation: NSABP, National Surgical Adjuvant Breast and Bowel Project.

Fig A1. Kaplan-Meier plots for proportion relapse free comparing treatment with tamoxifen (Tam) alone versus treatment with tamoxifen plus chemotherapy (Tam + chemo). (A) All patients; (B) low risk (recurrence score [RS] < 18); (C) intermediate risk (RS, 18-30); (D) high risk (RS ≥ 31). The number of patients at risk and the number of relapses (in parentheses) are provided below each part of the figure.
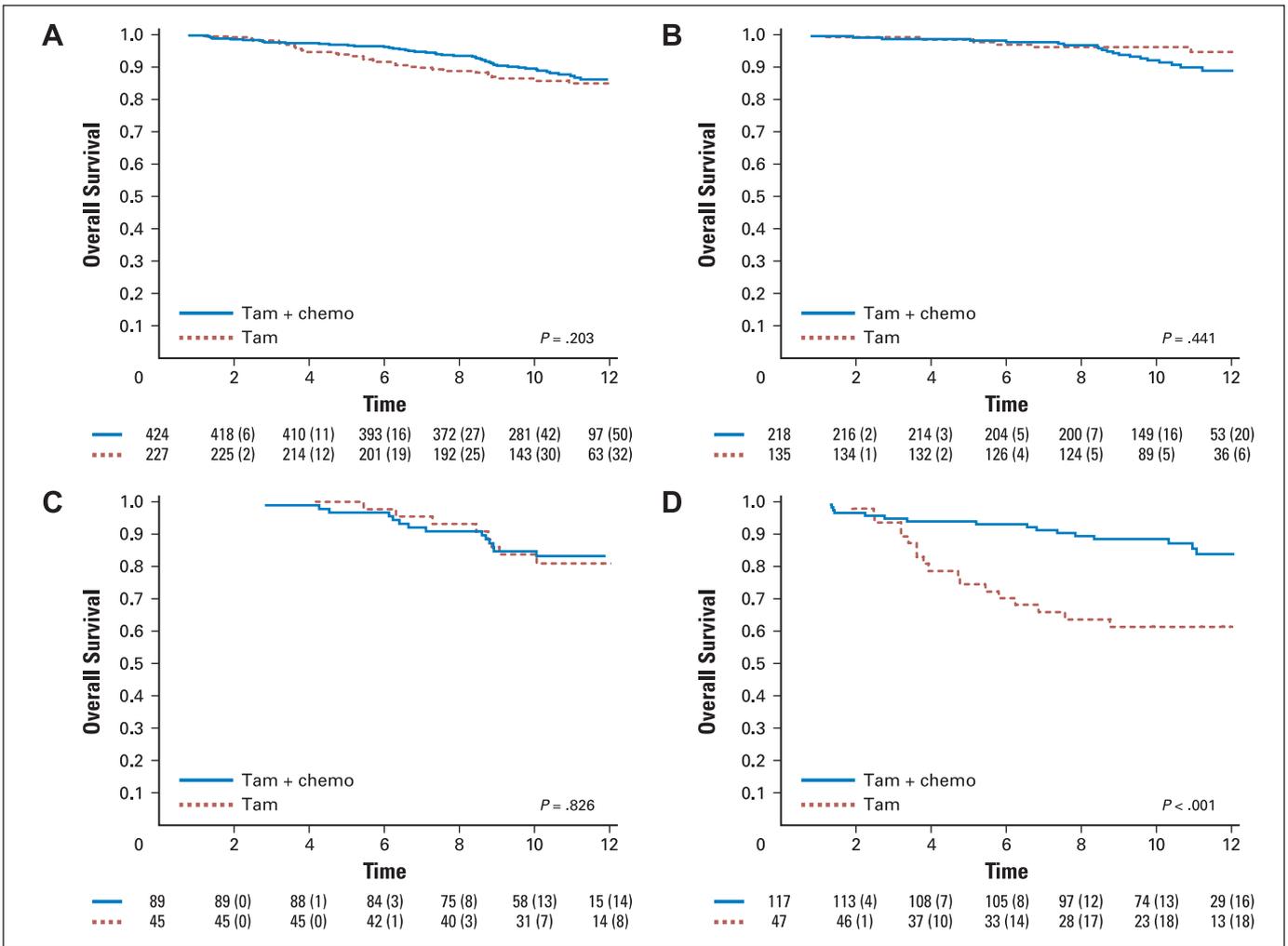
Fig A2. Kaplan-Meier plots for overall survival comparing treatment with tamoxifen (Tam) alone versus treatment with tamoxifen plus chemotherapy (Tam + chemo). (A) All patients; (B) low risk (recurrence score [RS] < 18); (C) intermediate risk (RS, 18-30); (D) high risk (RS ≥ 31). The number of patients at risk and the number of deaths (in parentheses) are provided below each part of the figure.

Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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GLOSSARY

RT-PCR (reverse-transcriptase polymerase chain reaction): PCR is a method that allows logarithmic amplification of short DNA sequences within a longer, double-stranded DNA molecule. Gene expression can be measured after extraction of total RNA and preparation of cDNA by a reverse-transcription step. Thus, RT-PCR enables the detection of PCR products on a real-time basis, making it a sensitive technique for quantitating changes in gene expression.

Gene expression profile: The expression of a set of genes in a biologic sample (eg, blood, tissue) using microarray, reverse-transcriptase polymerase chain reaction, or other technology capable of measuring gene expression.

Gene expression analysis: Technique for the simultaneous quantification of the mRNA expression level of thousands of genes. Can be performed using microarrays, reverse transcriptase polymerase chain reaction, or other technologies for measuring gene expression.

Recurrence Score: The Recurrence Score is a number between 0 and 100 that corresponds to a specific likelihood of breast cancer recurrence within 10 years of initial diagnosis. The score is derived from a mathematical function combining the expression values of 16 breast cancer–related genes and five reference genes.

21-gene reverse-transcriptase polymerase chain reaction assay: A 21-gene prognostic profile that has become a powerful predictor of outcomes in patients with breast cancer and outperforms currently used standard diagnostic criteria to predict development of future metastases and overall survival in patients with breast cancer. The 21-gene signature has been validated and is commercially available.

SCUBE2: Gene coding for signal peptide, CUB domain, EGF-like 2 protein, an endothelial cell–associated, secreted glycoprotein.

CTSL2 (cathepsin L2): A protein belonging to the papain family. *CTSL2* is the gene for a cysteine proteinase enzyme, which may function in protein turnover, antigen presentation, and bone remodeling.

Bcl2: A gene that codes for an antiapoptotic protein that protects cells from programmed cell death by preventing the activation of proapoptotic caspase proteins.