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ORIGINAL REPORT

Prognostic Impact of the Combination of Recurrence Score and Quantitative Estrogen Receptor Expression (*ESR1*) on Predicting Late Distant Recurrence Risk in Estrogen Receptor–Positive Breast Cancer After 5 Years of Tamoxifen: Results From NRG Oncology/National Surgical Adjuvant Breast and Bowel Project B-28 and B-14

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ABSTRACT

Purpose

We determined the utility of the 21-Gene Recurrence Score (RS) in predicting late (> 5 years) distant recurrence (LDR) in stage I and II breast cancer within high and low-*ESR1*–expressing groups.

Patients and Methods

RS was assessed in chemotherapy/tamoxifen-treated, estrogen receptor (ER) –positive, node-positive National Surgical Adjuvant Breast and Bowel Project B-28 patients and tamoxifen-treated, ER-positive, node-negative B-14 patients. The association of the RS with risk of distant recurrence (DR) 0 to 5 years and those at risk > 5 years was assessed. An *ESR1* expression cut point was optimized in B-28 and tested in B-14.

Results

Median follow-up was 11.2 years for B-28 and 13.9 years for B-14. Of 1,065 B-28 patients, 36% had low (< 18), 34% intermediate (18 to 30), and 30% high (≥ 31) RS. Of 668 B-14 patients, 51% had low, 22% intermediate, and 27% high RS. Median *ESR1* expression by reverse transcriptase polymerase chain reaction was: B-28, 9.7 normalized expression cycle threshold units (C_T) and B-14, 10.7 C_T. In B-28, RS was associated with DR 0 to 5 years (log-rank $P < .001$) and > 5 to 10 years (log-rank $P = .02$) regardless of *ESR1* expression. An *ESR1* expression cut point of 9.1 C_T was identified in B-28. It was validated in B-14 patients for whom the RS was associated with DR in years 5 to 15: 6.8% (95% CI, 4.4% to 10.6%) versus 11.2% (95% CI, 6.2% to 19.9%) versus 16.4% (95% CI, 10.2% to 25.7%) for RS < 18, RS 18 to 30, and RS ≥ 31, respectively (log-rank $P = .01$).

Conclusion

For LDR, RS is strongly prognostic in patients with higher quantitative *ESR1*. Risk of LDR is relatively low for patients with low RS. These results suggest the value of extended tamoxifen therapy merits evaluation in patients with intermediate and high RS with higher *ESR1* expression at initial diagnosis.

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INTRODUCTION

Estrogen receptor (ER) –positive breast cancer shows a protracted risk of recurrence, with approximately 50% of recurrences occurring after 5 years (late distant recurrence, LDR) in contrast to ER-negative breast cancer, which recurs primarily within the first 5 years.[1-4] ER-positive breast cancers at the greatest risk of LDR have been shown to have both high ER and high proliferation gene expression.[5] In ER-positive breast cancer, after 5 years of endocrine therapy, this time-dependent association between higher ER/ER-related gene expression, higher proliferation gene expression, and LDR was recently confirmed using quantitative assessments of ER and proliferation gene expression.[6]

This association is clinically relevant. Five years of adjuvant tamoxifen substantially reduces recurrence rates for at least 15 years after diagnosis.[1] National Surgical Adjuvant Breast and

Bowel Project (NSABP) B-14 evaluated 5 versus 10 years of tamoxifen and suggested that > 5 years of tamoxifen was not warranted, as patients treated with an additional 5 years of tamoxifen fared worse than those who received placebo.[7] In contrast, the ATLAS (Adjuvant Tamoxifen Longer Against Shorter) and aTTom (Adjuvant Tamoxifen—To Offer More?) trials showed that 10 years of tamoxifen significantly reduces risk of recurrence and breast cancer mortality compared with 5 years of treatment.[8,9] NCIC Clinical Trials Group MA17 also demonstrated that extended endocrine therapy with letrozole improves outcomes in postmenopausal patients with hormone receptor–positive breast cancer after 5 years of tamoxifen.[10] ASCO guidelines now recommend an extended 5 years of tamoxifen.[11] Although studies have shown that select clinicopathologic factors are associated with higher risk of LDR (eg, lymph node–positive disease or larger-sized tumors), there remains an unmet clinical need to more accurately identify ER-positive patients at greater risk of LDR given the adverse effects of extended hormone therapy.[12,13] Molecular assays may help identify patients who will benefit from prolonged hormonal therapy.[6,14-16] For hormone receptor–positive, early-stage breast cancer, the 21-Gene Recurrence Score assay (RS) is a widely used predictor of 10-year risk of DR[17-22] and of the likely benefit of adjuvant chemotherapy.[18,19] Individually reported quantitative estrogen receptor mRNA level (*ESR1*) is also a strong continuous predictor of tamoxifen benefit.[23,24] The findings of Dowsett et al[6] suggest that results of the RS assay may be useful in the prediction of LDR. Thus, the objective of this study was to determine if the RS can identify a group of patients with breast cancer who are at low risk for LDR and to determine if this relationship is dependent on levels of quantitative *ESR1* expression.

PATIENTS AND METHODS

Studies and Patients

Included were patients with RS information from NSABP B-14 (tamoxifen-only arm) and B-28[17,22] (Fig 1). B-14 compared placebo or tamoxifen (N = 2,892). The RS study included 668 ER-positive, tamoxifen-treated patients.[7,25,26] Median follow-up was 13.9 years. B-28 compared doxorubicin plus cyclophosphamide (AC; 4 cycles) with four cycles of AC followed by four cycles of paclitaxel (N = 3,060) with 5 years of tamoxifen for hormone receptor–positive patients.[27] The RS study included 1,065 ER-positive, tamoxifen-treated patients. Median follow-up was 11.2 years. RS and quantitative *ESR1* methodology were previously described.[17,28] Participating institutions obtained approval from their human investigations committee or institutional review board and filed assurances with the Department of Health and Human Services. Written informed consent was required for enrollment.

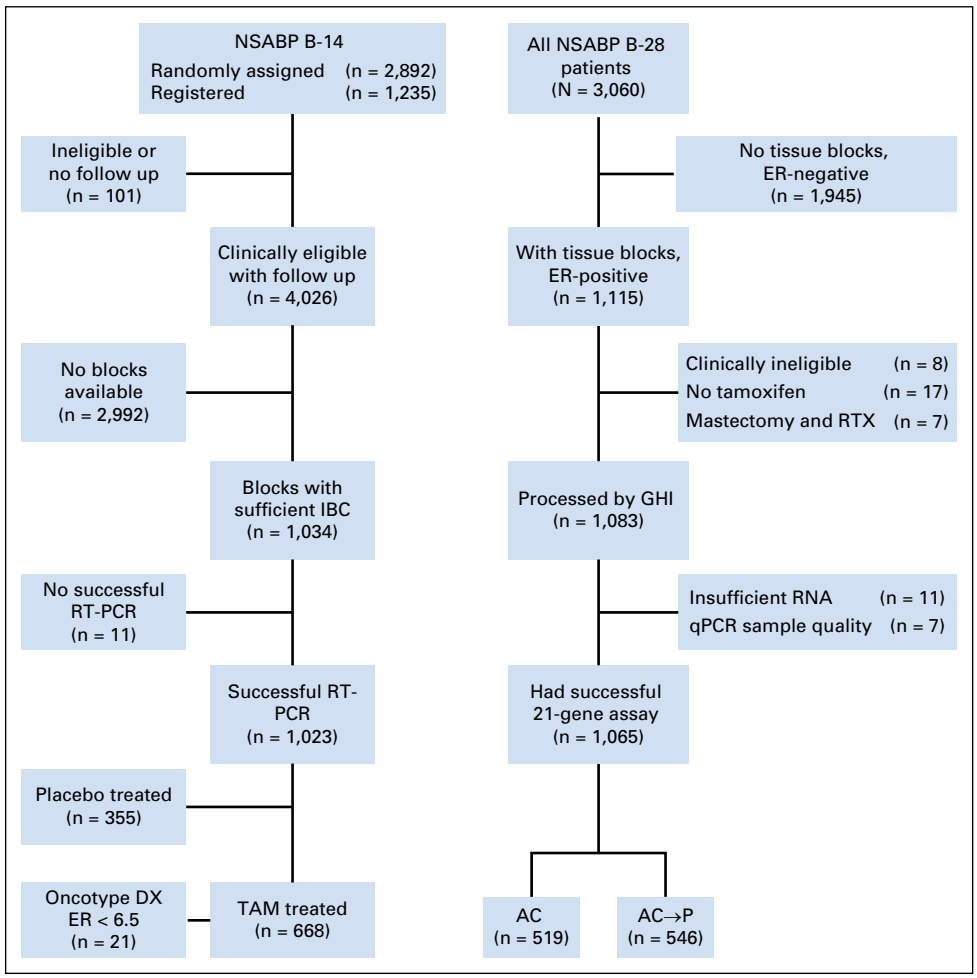

Fig 1. CONSORT diagram. AC, doxorubicin plus cyclophosphamide; AC→P, doxorubicin plus cyclophosphamide followed by paclitaxel; ER, estrogen receptor; GHI, Genomic Health, Inc.; IBC, invasive breast cancer; NSABP, National Surgical Adjuvant Breast and Bowel Project; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; RTX, radiotherapy; TAM, tamoxifen.

Statistical Analysis and Study End Points

The primary end point was distant recurrence-free interval, defined as time from study entry to first distant recurrence (DR), with contralateral breast cancer or non–breast second primary cancers ignored (B-28) or treated as censoring events (B-14), and death or loss to follow-up treated as a censoring event. Patients were grouped into low-RS (< 18), intermediate-RS (18 to 30), and high-RS (≥ 31) groups. The association between RS and DR by time period (0 to 5 years, > 5 years) was determined for all patients in B-28 and B-14 using Kaplan-Meier estimates and log-rank tests for the RS risk groups and Cox proportional hazards models for the continuous RS.

The development stage used B-28 to establish the *ESR1* mRNA expression cut point at a natural quantile of the distribution on the basis of the hazard ratio (HR) for the RS association with LDR risk (> 5 years) in high-*ESR1*–expressing patients, on the basis of Cox models with time-dependent effects. Subsequently, the cut point was independently tested in B-14. Analyses were conducted to determine the robustness of the results to the specific cut point given patient characteristic differences between the two studies. Kaplan-Meier estimates and log-rank tests were used to evaluate outcomes in the RS groups by time period (0 to 5 years, > 5 years) and *ESR1* expression level (lower or higher *ESR1* expression on the basis of the B-28 optimized cut point).

Log-rank statistics for 0 to 5 years are based on all data up to 5 years with administrative censoring at 5 years. Log-rank statistics after 5 years are based on all patients who were DR-free at 5 years and followed for recurrence after 5 years. Cox models assessed the association strength between the continuous RS and DR risk by time period and *ESR1* level. Model diagnostics were performed, and alternative functional forms were considered for the association of the RS with LDR risk in high-*ESR1* expressors, including natural spline models (two degrees of freedom) and quadratic models on log-hazard scale. Multivariable Cox proportional hazards models examined whether RS provides independent prognostic information for LDR in higher-*ESR1*–expressing patients. Cox model HRs for the RS are estimated for a 50-point difference, CIs use the Wald method, and *P* values are based on likelihood ratio tests. Statistical significance used a *P* value $\leq .05$. NSABP and Genomic Health Inc conducted analyses jointly.

RESULTS

Patient Characteristics

There were 1,065 ER-positive patients in NSABP B-28 and 668 tamoxifen-treated ER-positive patients in B-14 with sufficient tissue for RNA extraction and the RS assay. Patient characteristics were described previously.[17,22] Clinicopathologic characteristics are summarized in Appendix Table A1 (online only).

Of B-28 patients, 36% had low (< 18), 34% intermediate (18 to 30), and 30% high (≥ 31) RS results. Of B-14 patients, 51% had low, 22% intermediate, and 27% high RS results. The mean (standard deviation) reference normalized expression cycle threshold (C_T) levels for *ESR1* were 9.6 (1.2) in B-28 and 10.5 (1.7) in B-14. The association between the continuous RS and *ESR1* in each study is shown in Appendix Figure A1 (online only). Although there was a shift toward higher *ESR1* in B-14 compared with B-28, the association between the RS and *ESR1* level was similar in both studies.

Late Recurrence Events

In B-28, of 359 DR events, 168 (47%) occurred after 5 years. In B-14, of 109 DR events, 50 (46%) occurred after 5 years. Previous studies demonstrated the association between the RS and cumulative risk of recurrence over 10 years for both studies.[17,22] When divided by early DR or LDR, in B-28, the RS risk groups were prognostic for both early (0 to 5 years, log-rank $P < .001$) and late (> 5 years, log-rank $P = .02$) DR risk (Figs 2A and 2B). In B-14, RS risk groups were prognostic for early DR risk (LDR not statistically significant [log-rank $P = .06$; Figs 2C and 2D]).

ESR1 *Cut Point Selection in B-28*

B-28 was used to establish a quantitative *ESR1* expression cut point identifying a subgroup for which the RS predicted LDR. A range of cut points were evaluated based on the HR for the association of the continuous RS with LDR (Appendix Fig A2, online only). The first tertile (9.1 C_T units) was selected as the cut point to test for strength and precision of the HR estimate and to determine the size of the higher-*ESR1*–expressing patient population. HR estimates were robust to cut point choice, and the lower limit of the CI for the RS HR was above 1.0 for cut point values near 9.1. The association strength and CI width increased gradually with increasing values of the cut point above 9.1. For all subsequent results, higher *ESR1* expression is defined as expression > 9.1 C_T cut point.

Within the B-28 cohort, RS was associated with DR risk up to 5 years in lower and higher *ESR1* expression (Fig 3; Table 1; Appendix Fig A3, online only). After 5 years, the RS was associated with DR only in the higher-*ESR1*–expressing patients (log-rank $P = .001$ for RS risk groups) but not in the lower-*ESR1*–expressing patients (log-rank $P = .87$). In years 5 to 10, for higher-*ESR1*–expressing patients, the DR risks were 10.5% (95% CI, 7.3% to 14.8%) in low-RS, 22.5% (17.0% to 29.5%) in intermediate-RS, and 22.6% (15.6% to 32.0%) in the high-RS group.

Cox models for DR were fit with terms for the continuous RS, time period, *ESR1*-expression group, and the two- and three-way interactions among them. Interaction tests were conducted as secondary analyses on the basis of these models and were not the basis for cut point selection. The association between RS and LDR risk differed between lower- and higher-*ESR1*–expression groups, with a statistically significant two-way interaction between the RS and *ESR1*-expression group after 5 years ($P = .04$). The corresponding interaction term was not significant for early events (0 to 5 years, $P = .19$). In an analysis restricted to late events, the interaction term among *ESR1*(> 9.1 *v* < 9.1) and RS, indicating effect modification on the basis of an *ESR1* threshold at 9.1 after 5 years, remained statistically significant after adjusting for clinical and pathologic characteristics ($P = .02$).

ESR1 *Expression Cut Point Testing in B-14*

The 9.1 C_T cut point for *ESR1* expression was subsequently tested independently in the B-14 data set. Because the distribution of *ESR1* in B-14 was higher than B-28, the 9.1 C_T cut point was the 14th percentile in B-14, such that 86% of patients were in the higher *ESR1* group.

Among the higher-*ESR1*–expressing patients, the RS was a significant predictor of DR after 5 years (log-rank $P = .01$), confirming the B-28 results (Fig 4; Table 1). In years 5 to 10, for higher-*ESR1*–expressing patients, DR risks were 4.7% (2.8% to 8.0%) in the low, 4.1% (1.6% to 10.6%) in the intermediate, and 12.6% (7.4% to 21.2%) in the high-RS group. In years 5 to 15, for

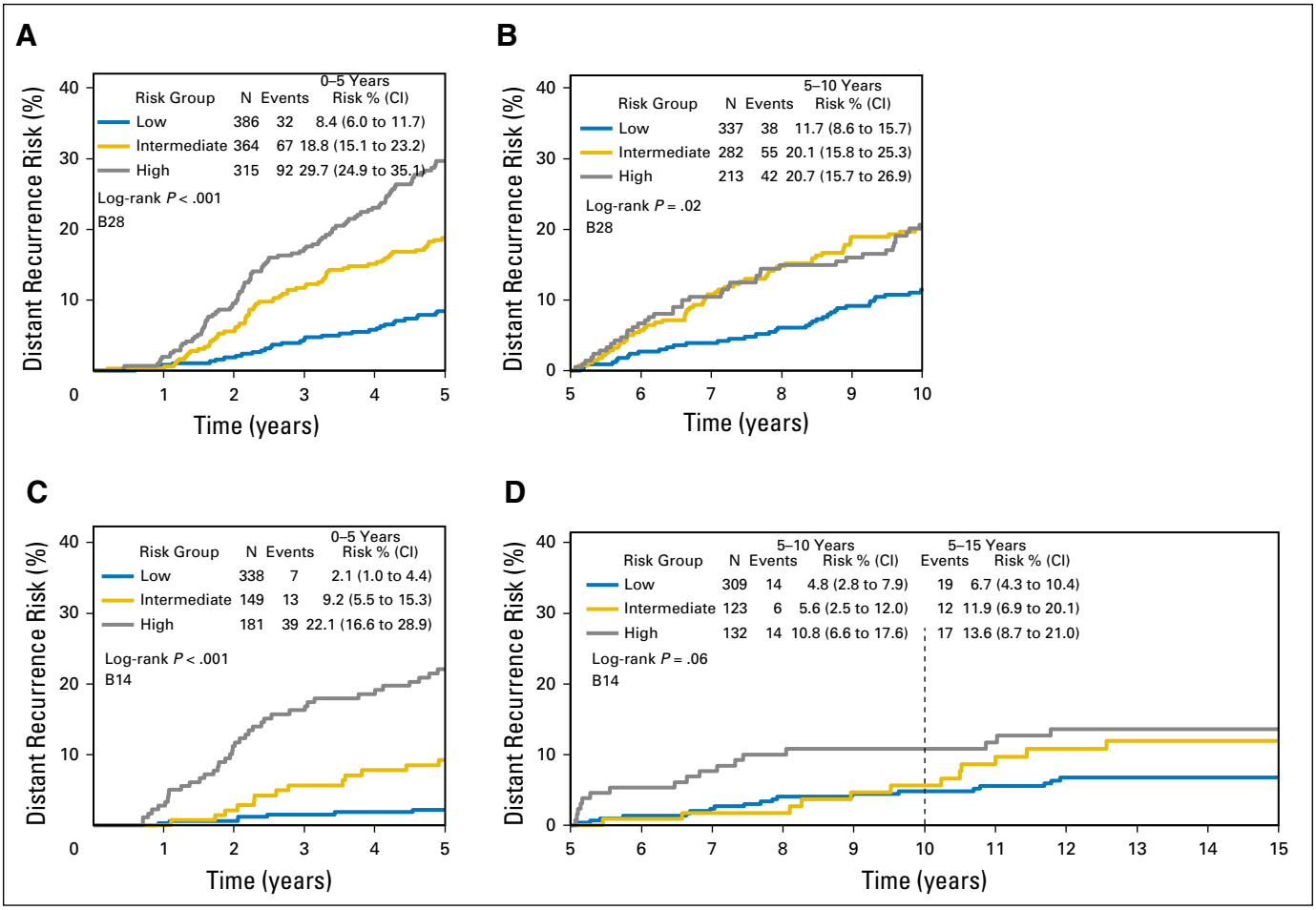

Fig 2. Kaplan-Meier curves and estimates for distant recurrence risk in B-28 and B-14 by recurrence score risk groups and time period. Event counts are for those occurring within the time period shown. (A) Curves for B-28, 0 to 5 years; (B) curves for B-28, 5 to 10 years; (C) curves for B-14, 0 to 5 years; (D) curves for B-14, 5 to 15 years.

higher-*ESR1*–expressing patients, the DR risks were 6.8% (4.4% to 10.6%) in the low, 11.2% (6.2% to 19.9%) in the intermediate, and 16.4% (10.2% to 25.7%) in the high-RS group (Appendix Figs A4 and A5, online only).

Similar to B-28 results, RS was associated with DR risk before 5 years regardless of *ESR1* expression level in B-14 (Table 1; Appendix Fig A4, online only). In B-14 there were few events after 5 years among the lower-*ESR1*–expressing patients, with most events occurring before 5 years in the high-RS patients with lower *ESR1*.

Cox model interaction tests were conducted as secondary analyses. The association between RS and LDR risk differed between lower- and higher-*ESR1* groups for late events (two-way interaction, $P = .03$). There was no statistical evidence of a two-way interaction for early events (0 to 5 years, $P = .35$). The significant interaction for late events persisted after adjustment for covariates ($P = .01$).

After successfully testing the a priori–determined 9.1 cut point, sensitivity analyses determined the robustness of the results to the specific *ESR1* cut point value. The RS HR was nearly constant for cut points between 8.7 and 9.7 (2.17 and 2.23), and the lower limit of the 95% CI excluded 1.0 for cut points 8.2 to 10.7. Successful testing of the established cut point was not overly sensitive to the specific value used. The HRs and CI widths increased gradually with increasing cut point values above 9.7, as the higher *ESR1* sample size decreased.

Subgroup Analyses

In B-28 and B-14, the RS risk groups' association with LDR risk for higher-*ESR1*–expressing patients was explored within subgroups according to clinicopathologic characteristics. This association was consistent with the overall results (Fig 5). In B-28, one to three positive nodes had a lower LDR risk compared with four or more positive nodes (Appendix Table A2, online only). In patients with a low RS, the risk of DR between 5 and 10 years was 7.9% (4.8% to 12.8%) in patients with one to three positive nodes, compared with 16.7% (10.0% to 27.0%) in patients with four or more positive nodes. For *ERBB2*-negative or equivocal patients, including n = 937 (88.0%) in B-28 and n = 594 (88.9%) in B-14, the association of the RS with LDR risk in higher-*ESR1*–expressing patients was similar to the overall results (data not shown).

Functional Form Assessment

Alternative functional forms for the association of the RS with LDR risk in high-*ESR1*–expressing patients were explored in B-14

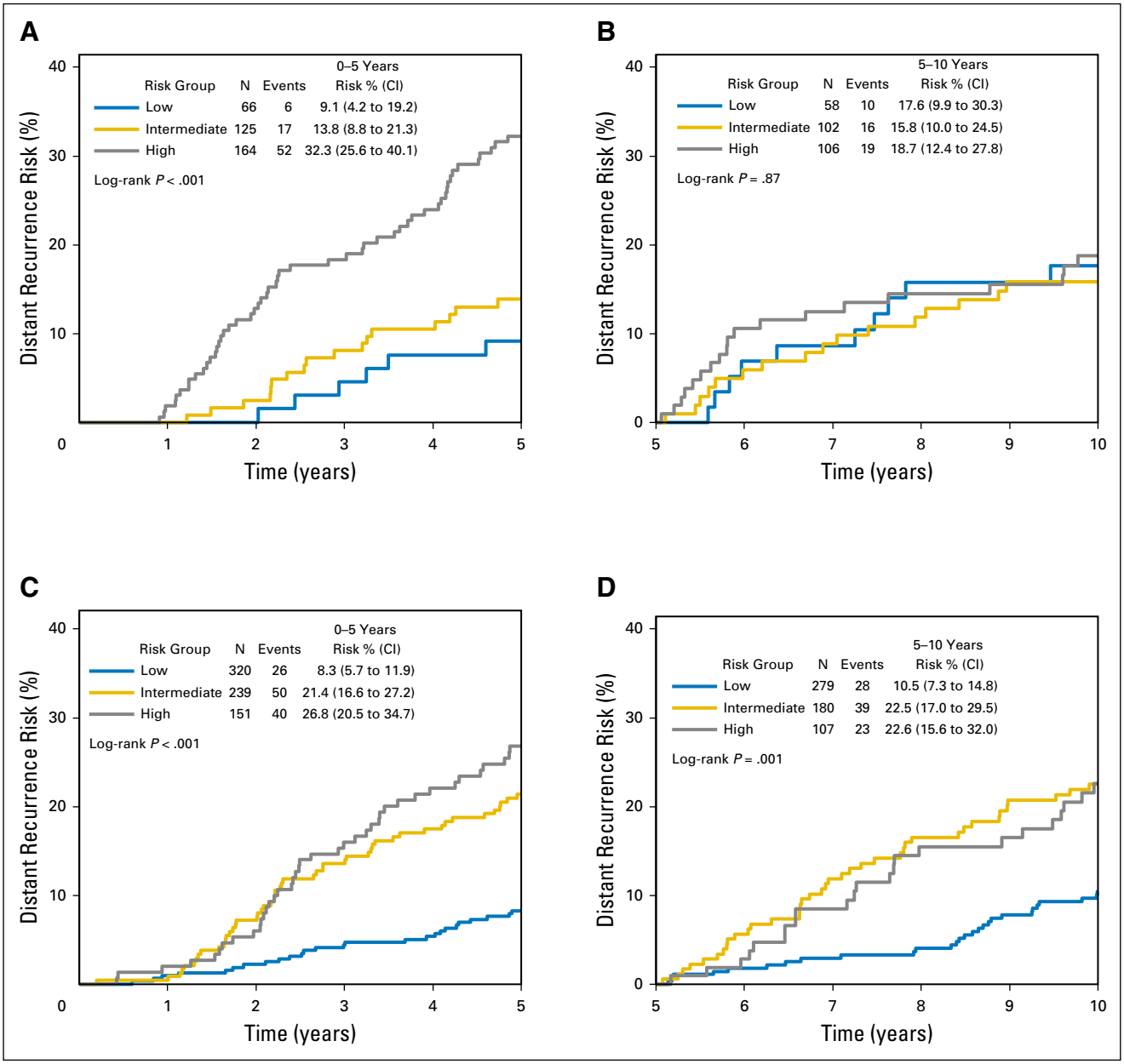

Fig 3. Kaplan-Meier curves and estimates for distant recurrence risk in B-28 by recurrence score risk groups, *ESR1* expression level, and time period. (A) Curves for *ESR1* ≤ 9.1, time 0 to 5 years; (B) curves for *ESR1* ≤ 9.1, time 5 to 10 years; (C) curves for *ESR1* > 9.1, time 0 to 5 years; (D) curves for *ESR1* > 9.1, time 5 to 10 years.

and B-28. In B-28, the linear model provided a good fit. In B-14, a better fit was provided by a quadratic model ($P < .001$ for association of the RS with DR risk; $P < .001$ for the test of whether the additional quadratic term provided a better fit than a linear term alone).[23] Appendix Figure A6 (online only) shows the estimated association between the RS and LDR risk in high-*ESR1*–expressing patients.

Multivariable Models

In B-28, only nodes ($P \leq .001$) and continuous RS ($P = .004$) were significant predictors of LDR in higher-*ESR1*–expressing patients in a multivariable model adjusting for nodes, size, age, grade, surgery type, and treatment (Appendix Table A2, online only). In B-14, only central grade ($P = .003$) and continuous RS ($P = .005$; quadratic model, which provided a significantly better fit) were significant predictors of LDR in higher-*ESR1*–expressing patients in a multivariable model after adjustment for age, size, and grade (Appendix Table A3, online only).[23]

In B-14, patients completing 5 years of tamoxifen were randomly assigned to an additional 5 years of treatment, although the extended tamoxifen was stopped early (median of 2.5 years; range, 1.6 to 5.0 years). To determine if extended tamoxifen treatment affected results for high-*ESR1*–expressing patients, the association of the RS with LDR in the high-*ESR1*–expressing patients who were re-randomly assigned to extended tamoxifen or

Table 1. Association of the Continuous Recurrence Score With Distant Recurrence Risk From 0 to 5 Years and After 5 Years According to *ESR1* Expression in B-28 and B-14, on the Basis of Cox Proportional Hazards Models

ESR1 Expression Group	0 to 5 Years				After 5 Years			
	No.	Events	HR (95% CI)*	*P**	No.†	Events	HR (95% CI)*	*P**
NSABP B-28 ER-positive patients (n = 1,065)								
All patients	1,065	191	4.22 (2.93 to 6.07)	< .001	832	168	1.66 (1.05 to 2.61)	.04
ESR1 ≤ 9.1	355	75	5.86 (3.18 to 10.80)	< .001	266	52	0.85 (0.36 to 2.00)	.70
ESR1 > 9.1	710	116	3.46 (2.09 to 5.71)	< .001	566	116	2.43 (1.42 to 4.18)	.003
NSABP B-14 tamoxifen-treated patients (n = 668)								
All patients	668	59	6.04 (3.88 to 9.41)	< .001	564	50	1.55 (0.81 to 2.97)	.20
ESR1 ≤ 9.1	91	19	4.29 (1.86 to 9.89)	< .001	67	6	0.21 (0.02 to 2.33)	.14
ESR1 > 9.1	577	40	5.85 (3.23 to 10.60)	< .001	497	44	2.23 (1.11 to 4.47)	.04

Abbreviations: ER, estrogen receptor; HR, hazard ratio; NSABP, National Surgical Adjuvant Breast and Bowel Project; RS, recurrence score.
*On the basis of Cox proportional hazards models that include the RS with time dependence (all patients' results) or the RS, ER group indicator, the RS by ER interaction, and time dependence for each parameter (*ESR1* expression group results). RS HRs are presented for a 50-point difference. *P* values are from likelihood ratio tests; CIs use the Wald method. Interaction *P* values were not the primary basis of cut point selection or testing but were as follows: RS × *ESR1* expression group after 5 years: *P* = .04 (B-28) and *P* = .03 (B-14). RS × *ESR1* expression group 0 to 5 years: *P* = .19 (B-28) and *P* = .35 (B-14). The interactions after 5 years remained significant (*P* < .05) when adjusting for age, nodal status (B-28 only), grade, and size in the models.
†Patients at risk at 5 years.

placebo was assessed. For the 144 high-*ESR1*–expressing patients at risk after 5 years who were among those assigned to extended tamoxifen, the DR risk estimates over 5 to 15 years for the low-, intermediate-, and high-RS groups were 6.9% (3.1% to 14.7%), 5.0% (0.7% to 30.5%), and 17.5% (7.7% to 37.2%).

DISCUSSION

Using the RS assay, we tested patient tumor tissue from NSABP B-28 and found that the RS and quantitative *ESR1* cutoff of 9.1 C_T were able to quantify the likelihood of LDR in patients with node-positive, ER-positive breast cancer treated with 5 years of chemotherapy plus tamoxifen. We subsequently independently tested and confirmed the quantitative *ESR1* expression cut point of 9.1 C_T and RS as an independent predictor of LDR in patients with node-negative, ER-positive breast cancer treated with tamoxifen in B-14. Because the distribution of *ESR1* expression in B-14 was generally higher than that of B-28, the majority of B-14 patients were in the higher-*ESR1*–expression subset. In the lower-*ESR1*–expression group the RS was a significant predictor of early DR but was not a significant predictor of LDR; however, few events were experienced after 5 years among the lower-*ESR1*–expressing patients, for whom most events occurred before 5 years in the overly represented high-RS patients, consistent with ER-poor biology.[5]

These data have implications with respect to cancer pathogenesis and metastasis. Our data confirm and extend results of the foundational gene expression studies, starting with Bianchini et al,[5] who used gene expression arrays to show that highly proliferative, high-ER gene–expressing tumors are at the greatest risk of late relapse. Dowsett et al[6] further confirmed this association using the ER and proliferation gene expression modules in ER-positive, human epidermal growth factor receptor 2–negative tumors from the Arimidex, Tamoxifen, Alone or Combined (ATAC) randomized clinical trial. In the current study, we have identified a statistically significant interaction between quantitative *ESR1* expression, the RS (which includes the five-gene proliferation group), and the risk of LDR in B-28 (interaction *P* = .04) and B-14 (*P* = .03). Similar results were seen for the ER and proliferation modules alone (data not shown). In the *ESR1*-poor group, predominantly populated by high-RS patients, the RS was strongly

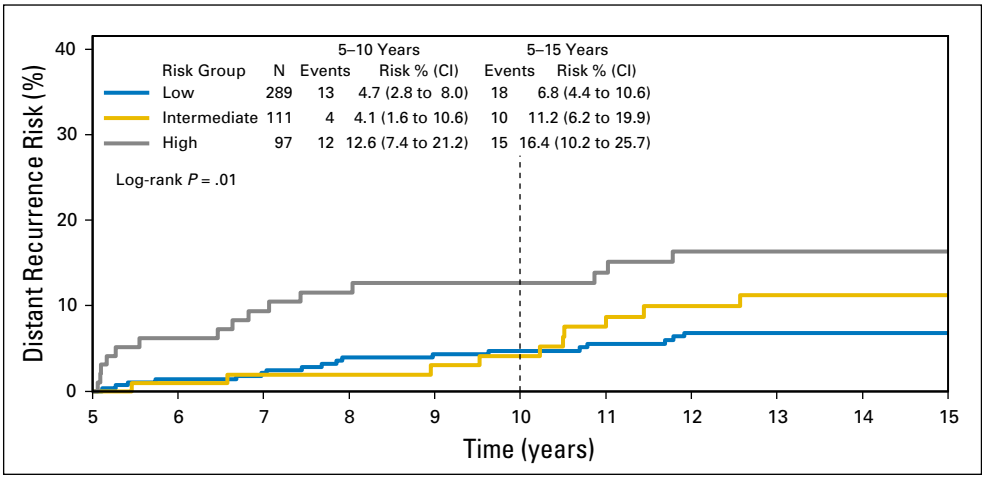

Fig 4. Kaplan-Meier curves and estimates for distant recurrence risk over 5 to 15 years, by recurrence score risk groups, in high-*ESR1*–expressing patients in B-14.

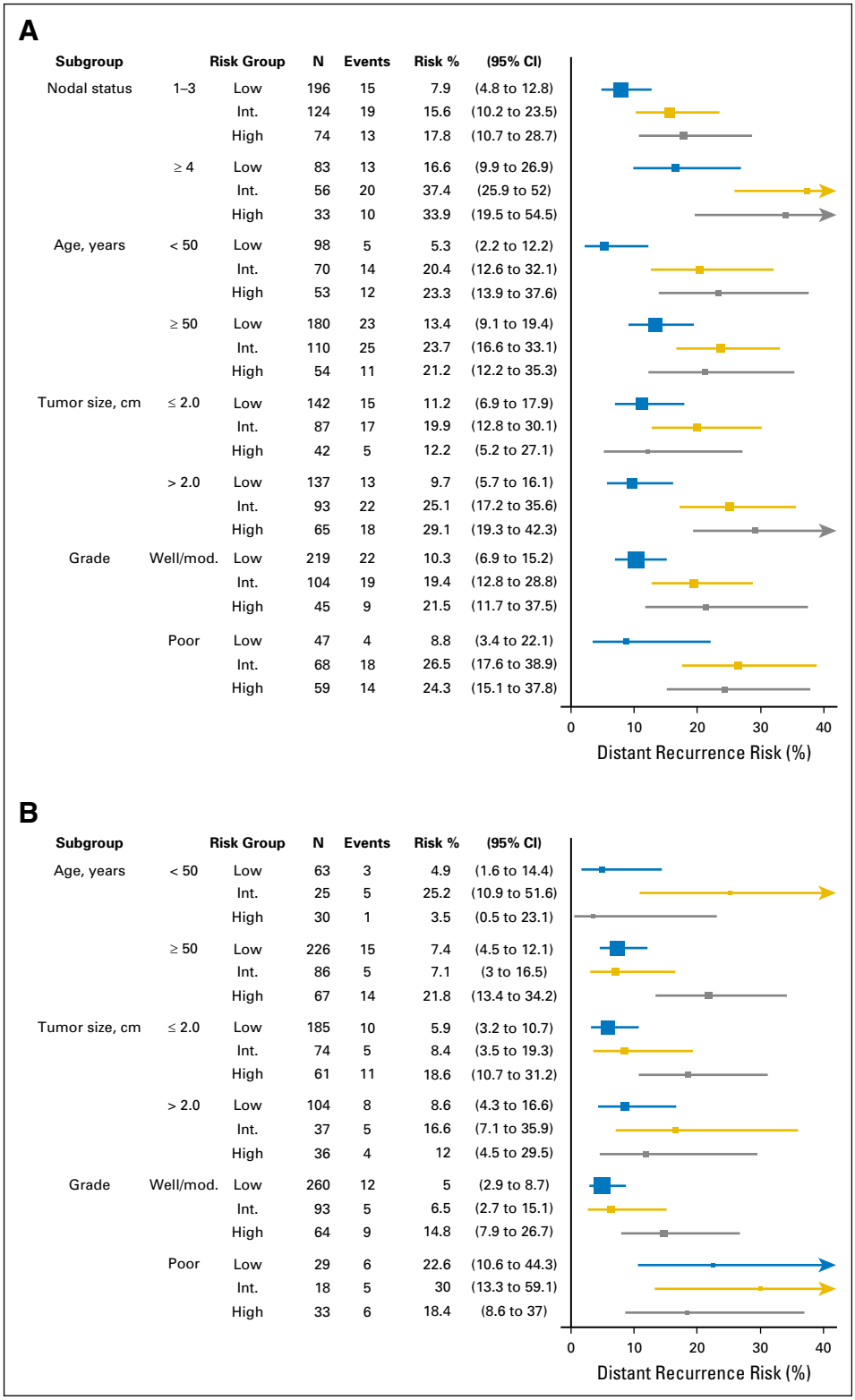

Fig 5. Forest plots with Kaplan-Meier estimates and 95% CIs for the risk of distant recurrence after 5 years by recurrence score risk group in high-*ESR1*–expressing patients, in (A) B-28 (from 5 to 10 years) and (B) B-14 (from 5 to 15 years), within subgroups according to clinical and pathology characteristics. Int, intermediate; Mod, moderate

associated with the risk of early DR, suggesting that early relapses are most common in tumors intrinsically resistant to endocrine treatment.[23] Alternatively, *ESR1*-rich tumors are at risk for both early DR and LDR, and the RS risk groups provided further risk stratification. Although underpowered in B-28 and B-14, the results in the higher-*ESR1*–expressing patients are consistent across subgroups.

Previous reports have suggested that the RS does not predict LDR, whereas other multigene expression–based assays do.[14-16] Although the number of late events is clinically important, the

numbers are relatively small, and reports of comparisons of the RS with other tests show differences with overlapping CIs.[16,29]

How do these results and other studies of LDR risk affect the extended endocrine therapy treatment decision? They confirm studies of other molecular assays in postmenopausal patients and extend these findings to premenopausal women: at-risk patients have varying rates of LDR, and a low-risk group with less than a 5% risk of recurrence in the second quinquennium can be identified.[15,16,30-32] In addition to nodal status,[27] there seems to be additional discrimination of LDR risk by genomic assays that combine measures of proliferation and *ESR1* expression or ER gene groups.[5,14,15,30] Recent ASCO guidelines, on evidence derived from five studies of tamoxifen treatment beyond 5 years, recommend extended treatment of all hormone receptor–positive patients.[11] Use of the RS, which includes the proliferation gene module and quantitative *ESR1* expression for risk of LDR assessment, may be useful for patient risk stratification, and these results are already available for thousands of patients for whom the RS was used on initial diagnosis. Additional studies are needed to validate that genomic factors can predict which patients should be treated with only 5 years of hormonal therapy.

The strengths of this study include that these refined estimates of LDR can be derived from RS and quantitative *ESR1* gene expression results. The present data are from randomized, well-controlled studies with sufficient numbers of LDR events for effective cut point selection and testing. Our analyses include an independent training population and separate, independent test population. There are also limitations. This report cannot directly address the question of which subset of patients derive benefit from $>$ 5 years of hormonal treatment. Also, more contemporary study outcomes have improved over time; for example, the total rate of DR at 9 years in node-negative patients treated with tamoxifen or anastrozole with low-RS results (n = 872) was $<$ 4% in TransATAC (Translational Arimidex, Tamoxifen, Alone or in Combination).[20]

The RS alone is a significant prognostic factor for cumulative risk over 10 years, although this association is attenuated in later years. For LDR, the RS is prognostic in the majority of ER-positive, lymph node–negative patients with higher quantitative *ESR1* expression, where the risk of LDR is relatively low for patients with low-RS results, and these results confirm and extend the association between high proliferation and ER levels with LDR.[4,5,33] These RS and quantitative *ESR1* results may help select patients who could benefit most from hormonal therapy beyond 5 years of treatment and merit further study in larger cohorts, such as MA-17R and NSABP B-42.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Administrative support: Norman Wolmark
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Prognostic Impact of the Combination of Recurrence Score and Quantitative Estrogen Receptor Expression (*ESR1*) on Predicting Late Distant Recurrence Risk in Estrogen Receptor–Positive Breast Cancer After 5 Years of Tamoxifen: Results from NRG Oncology/National Surgical Adjuvant Breast and Bowel Project B-28 and B-14

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Appendix

Table A1. Characteristics of NSABP B-28 and B-14 Patients Included in the Current Study

Variable	No.	%
NSABP B-28 ER+ patients (n = 1065)		
Age, years		
< 50	511	48.0
≥ 50	554	52.0
Tumor size, cm		
≤ 2.0	483	45.4
2.1-4.0	467	43.8
≥ 4.1	115	10.8
Positive nodes, No.		
1-3	722	67.8
4-9	300	28.2
≥ 10	43	4.0
Central tumor grade		
Well	120	11.3
Moderate	499	46.9
Poor	405	38.0
Unknown	41	3.8
Treatment		
AC	519	48.7
AC→P	546	51.3
Surgery type		
Lumpectomy	461	43.3
Mastectomy	604	56.7
NSABP B-14 tamoxifen-treated patients (n = 668)		
Age, years		
< 50	194	29.0
≥ 50	474	71.0
Tumor size, cm		
≤ 2.0	414	62.0
2.1-4.0	220	32.9
≥ 4.1	34	5.0
Central tumor grade		
Well	224	33.5
Moderate	296	44.3
Poor	148	22.2

Abbreviations: AC, doxorubicin plus cyclophosphamide, AC→P, doxorubicin plus cyclophosphamide followed by paclitaxel; ER, estrogen receptor; NSABP, National Surgical Adjuvant Breast and Bowel Project.

Table A2. Late Recurrences Analyses Supportive Materials: Univariable and Multivariable Results for NSABP B-28.

	Univariable Models		Multivariable Model*	
Variable	HR (95% CI)†	*P*†	HR (95% CI)†	*P*†
Age ≥ 50 years	1.11 (0.76 to 1.62)	0.58	1.01 (0.68 to 1.49)	0.97
≥ 4 Positive nodes	2.15 (1.49 to 3.10)	< .001	2.11 (1.43 to 3.10)	< .001
Tumor size > 2 cm	1.20 (0.83 to 1.73)	0.34	1.12 (0.76 to 1.65)	0.57
Grade		0.04		0.32
Moderate *v* low	1.81 (0.90 to 3.64)		1.59 (0.79 to 3.22)	
Poor *v* low	2.34 (1.14 to 4.79)		1.70 (0.81 to 3.57)	
Treatment AC→P	1.07 (0.74 to 1.55)	0.71	1.03 (0.71 to 1.50)	0.87
Mastectomy	1.10 (0.77 to 1.59)	0.60	0.91 (0.62 to 1.34)	0.64
RS‡	2.45 (1.43 to 4.21)	0.002	2.46 (1.37 to 4.43)	0.005

NOTE: Association of clinical and pathology characteristics and the continuous RS with distant recurrence risk after 5 years in patients with higher *ESR1* expression (*ESR1* > 9.1) in B-28, in univariable and multivariable Cox proportional hazards models.

Abbreviations: AC→P, doxorubicin plus cyclophosphamide followed by paclitaxel; HR, hazard ratio; NSABP, National Surgical Adjuvant Breast and Bowel Project; RS, recurrence score.

*Age, tumor size, grade, treatment, and surgery type were not statistically significant in the multivariable models. Similar results were seen for the RS when adjusted for only the significant independent predictors.

†Based on Cox proportional hazards models of distant recurrence after 5 years in patients with higher *ESR1* expression. *P* values are from likelihood ratio tests; CIs use the Wald method.

‡RS hazard ratio presented for a 50-point difference.

Table A3. Late Recurrence Analyses Supportive Materials: Univariable and Multivariable Results for NSABP B-14

	Univariable Models		Multivariable Model*	
Variable	HR (95% CI)†	*P*†	HR (95% CI)†	*P*†
Age ≥ 50	1.22 (0.59 to 2.55)	.58	1.50 (0.71 to 3.17)	.27
Tumor size > 2 cm	1.22 (0.66 to 2.24)	.53	1.21 (0.65 to 2.23)	.55
Grade		< .001		.003
Moderate *v* low	2.25 (0.95 to 5.35)		1.75 (0.72 to 4.25)	
Poor *v* low	5.84 (2.42 to 14.09)		4.51 (1.78 to 11.44)	
RS‡	4.06 (1.91 to 8.62)	< .001	2.74 (1.25 to 6.00)	.004

NOTE: Association of clinical and pathology characteristics and the continuous RS with distant recurrence risk after 5 years in patients with higher *ESR1* expression (*ESR1* > 9.1) in B-14, in univariable and multivariable Cox proportional hazards models.

Abbreviations: AC→P, doxorubicin plus cyclophosphamide followed by paclitaxel; HR, hazard ratio; NSABP, National Surgical Adjuvant Breast and Bowel Project; RS, recurrence score.

*Age and tumor size were not statistically significant in the multivariable models. Similar results were seen for the RS when adjusted for only the significant independent predictors.

†Based on Cox proportional hazards models of distant recurrence after 5 years in patients with higher *ESR1* expression. *P* values are from likelihood ratio tests; confidence intervals use the Wald method.

‡HR estimated for RS = 40 versus RS = 10 based on the quadratic model, which includes linear and quadratic terms. The hypothesis test is for including both terms versus neither term (two degrees of freedom). See Figure A6 for a graphical depiction of the association between the RS and distant recurrence risk after 5 to 15 years.

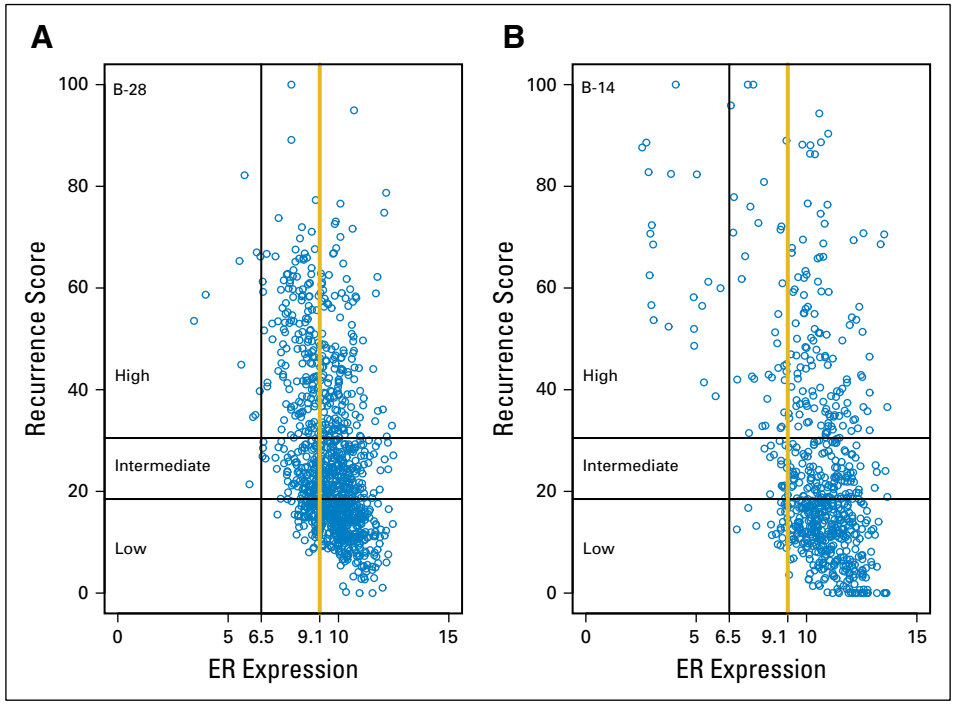

Fig A1. Scatter plots of recurrence score values by *ESR1* expression level in NSABP B-28 and B-14. Reference lines for *ESR1* expression are at the cut point for positivity by RT-PCR (6.5 C_T) and at the cut point identified in B-28 (9.1 C_T). (A) Plot for B-28; (B) Plot for B-14. ER, estrogen receptor.

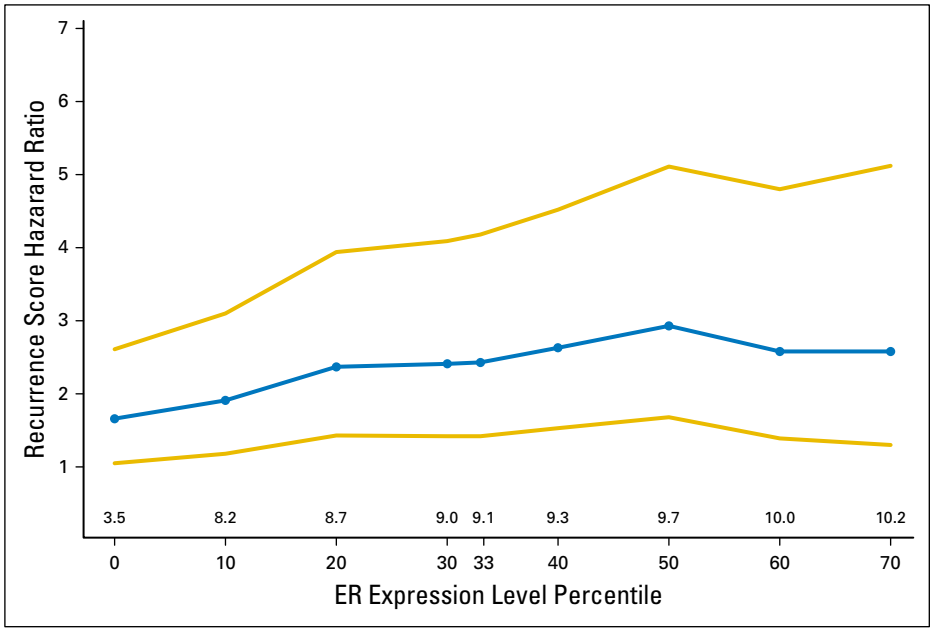

Fig A2. Cox model hazard ratios (and 95% CIs) for the continuous recurrence score as a predictor of distant recurrence risk in NSABP B-28, in patients with high *ESR1* expression, for a range of cut point values. The x-axis is the percentile of the *ESR1* distribution in NSABP B-28, annotated with the *ESR1* cut point value. ER, estrogen receptor.

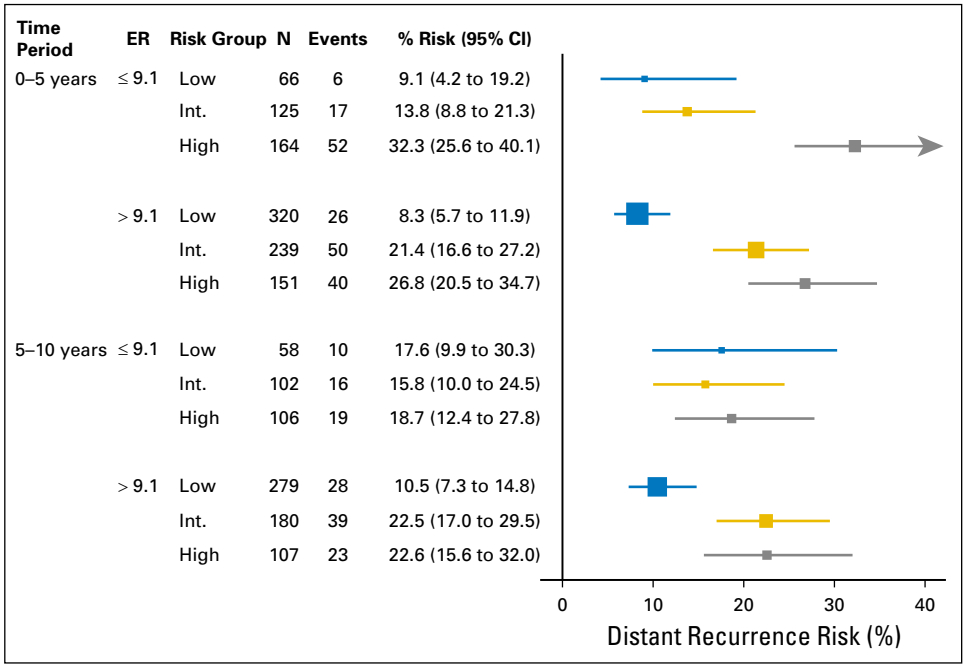

Fig A3. Forest plot with Kaplan-Meier estimates and 95% CIs for distant recurrence risk by recurrence score risk group, by *ESR1* expression level and time period, in NSABP B-28 patients. ER, estrogen receptor.

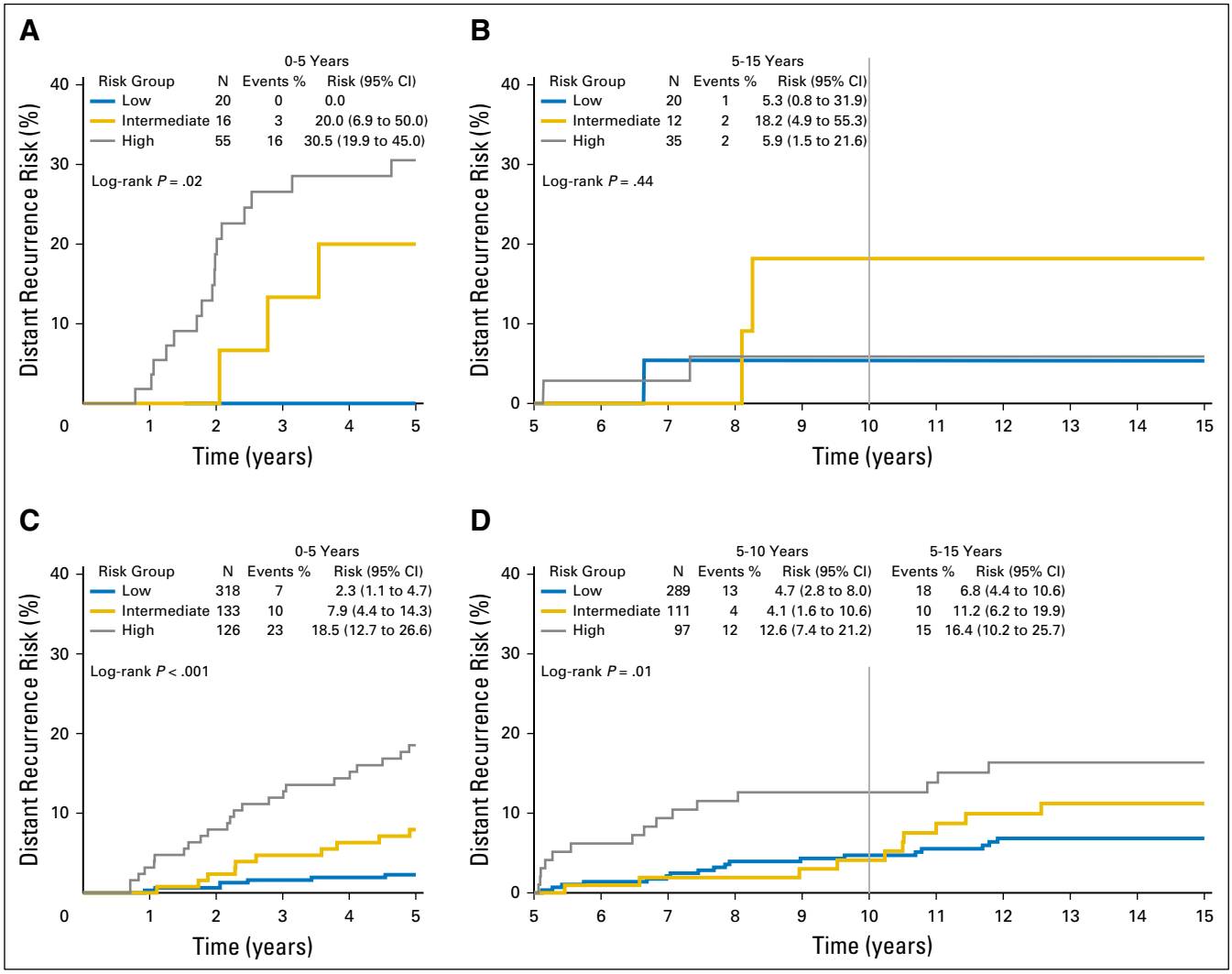

Fig A4. Kaplan-Meier curves and estimates for distant recurrence risk in NSABP B-14 by recurrence score risk groups, *ESR1* expression level, and time period. (A) Curves for *ESR1* ≤ 9.1, time 0-5 years; (B) Curves for *ESR1* ≤ 9.1, time 5-15 years; (C) Curves for *ESR1* > 9.1, time 0-5 years; (D) Curves for *ESR1* > 9.1, time 5-15 years.

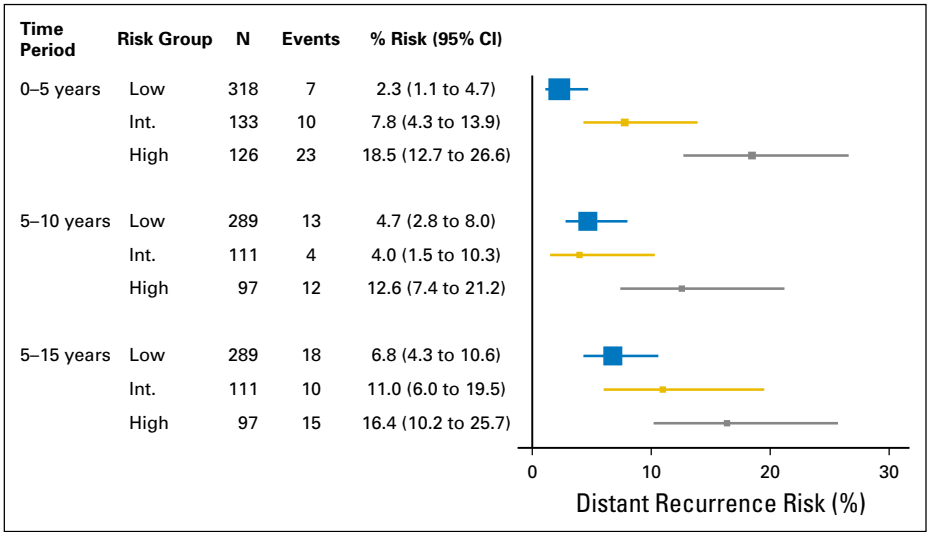

Fig A5. Forest plot with Kaplan-Meier estimates and 95% CIs for distant recurrence risk by recurrence score risk group, in high *ESR1*-expressing patients in NSABP B-14, by time period 0-5, 5-10, or 5-15 years.

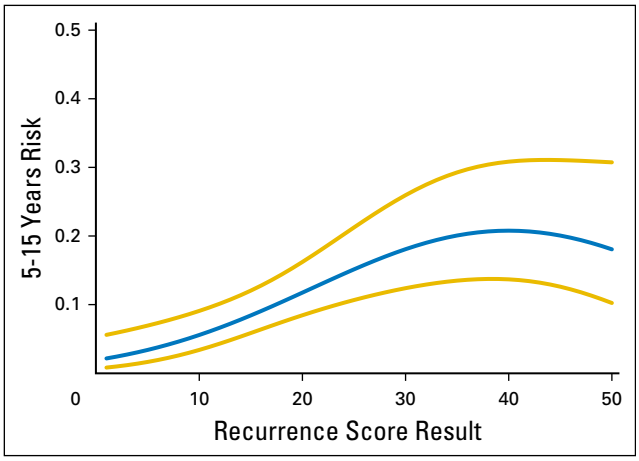

Fig A6. Distant recurrence risk estimates over years 5-15, with 95% CIs, in NSABP B-14 high-*ESR1*-expressing patients, as a function of the recurrence score. The estimates are based on the quadratic model.