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ARTICLE

# A Multigene Expression Assay to Predict Local Recurrence Risk for Ductal Carcinoma In Situ of the Breast

Lawrence J. Solin, Robert Gray, Frederick L. Baehner, Steven M. Butler, Lorie L. Hughes, Carl Yoshizawa, Diana B. Cherbavaz, Steven Shak, David L. Page, George W. Sledge Jr, Nancy E. Davidson, James N. Ingle, Edith A. Perez, William C. Wood, Joseph A. Sparano, Sunil Badve

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**Correspondence to:** Lawrence J. Solin, MD, FACR, FASTRO, Department of Radiation Oncology, Albert Einstein Medical Center, 5501 Old York Rd, Philadelphia, PA 19141 (e-mail: solin@einstein.edu).

**Background** For women with ductal carcinoma in situ (DCIS) of the breast, the risk of developing an ipsilateral breast event (IBE; defined as local recurrence of DCIS or invasive carcinoma) after surgical excision without radiation is not well defined by clinical and pathologic characteristics.

**Methods** The Onco*type* DX breast cancer assay was performed for patients with DCIS treated with surgical excision without radiation in the Eastern Cooperative Oncology Group (ECOG) E5194 study. The association of the prospectively defined DCIS Score (calculated from seven cancer-related genes and five reference genes) with the risk of developing an IBE was analyzed using Cox regression. All statistical tests were two-sided.

**Results** There were 327 patients with adequate tissue for analysis. The continuous DCIS Score was statistically significantly associated with the risk of developing an IBE (hazard ratio [HR] = 2.31, 95% confidence interval [CI] = 1.15 to 4.49; $P = .02$) when adjusted for tamoxifen use (prespecified primary analysis) and with invasive IBE (unadjusted HR = 3.68, 95% CI = 1.34 to 9.62; $P = .01$). For the prespecified DCIS risk groups of low, intermediate, and high, the 10-year risks of developing an IBE were 10.6%, 26.7%, and 25.9%, respectively, and for an invasive IBE, 3.7%, 12.3%, and 19.2%, respectively (both log rank $P \leq .006$). In multivariable analyses, factors associated with IBE risk were DCIS Score, tumor size, and menopausal status (all $P \leq .02$).

**Conclusions** The DCIS Score quantifies IBE risk and invasive IBE risk, complements traditional clinical and pathologic factors, and provides a new clinical tool to improve selecting individualized treatment for women with DCIS who meet the ECOG E5194 criteria.

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The treatment of ductal carcinoma in situ (DCIS; intraductal carcinoma) of the breast is variable, with concerns about both overtreatment and undertreatment (1–4). DCIS is commonly detected on screening mammography in the asymptomatic woman. Most women with newly diagnosed DCIS are eligible for breast conservation surgery (ie, excision or lumpectomy), either with or without radiation treatment. Randomized clinical trials have demonstrated that adding radiation treatment after surgical excision reduces the risk of developing local recurrence and invasive local recurrence by approximately 50% (5–14). However, most patients will not develop local recurrence if treated using surgical excision without radiation, and many patients are treated in contemporary practice using surgical excision alone (3,5–12,14–17).

Several studies have used clinical and pathologic features to attempt to define patients at low risk after surgical excision without radiation, including a prospective trial conducted by the Eastern Cooperative Oncology Group (ECOG) E5194 (18–21). However, reproducible and reliable methods using clinical and pathologic factors to select patients for surgical excision alone have not been established. The 2009 National Institutes of Health State-of-the-Science Conference included the research recommendation to develop and validate risk stratification models for identifying patients who may be managed with less therapeutic intervention (1).

This study was performed to determine whether the prospectively defined, 12-gene Onco*type* DX DCIS Score (hereafter referred to as the DCIS Score) quantifies local recurrence risk and provides risk information independent of traditional clinical and pathologic characteristics.

## Methods

### Development of the DCIS Score

A multistep strategy was used to develop and to validate the DCIS Score (see Supplementary Methods, available online, for additional details). The overall study was conducted using a rigorous prospective–retrospective design, which is a research methodology

that provides a high level of evidence supporting the validity of a tumor biomarker (22). Because of the paucity of DCIS study populations from major studies that included adequate numbers of tumor blocks with negative surgical margins, ECOG E5194 tissue specimens were preserved for the independent clinical validation component of this study.

Five developmental datasets were used to design the DCIS Score (Supplementary Table 1, available online) (23–27). These developmental datasets included studies of 1) either DCIS only or both DCIS and invasive breast carcinoma, but without clinical outcome data; or 2) invasive breast carcinoma with clinical outcome data. These datasets did not include ECOG E5194 tumor specimens.

The development of the DCIS Score was based in part on evidence that quantitative expression of genes from the 21-gene Onco*type* DX Recurrence Score (hereafter referred to as the Recurrence Score) may be useful for predicting local recurrence in DCIS. Two developmental studies without clinical outcomes showed a wide range of Recurrence Score values for DCIS. The first study compared expression levels of individual genes and Recurrence Scores for 30 patients with microdissected DCIS and invasive carcinoma when both were present within the same formalin-fixed paraffin-embedded tumor block (26). A strong correlation between gene expression levels of adjacent invasive and DCIS components was observed. The second study examined 96 DCIS specimens provided by Marin General Hospital, Greenbrae, California (Supplementary Tables 1 and 2, available online), and showed a similar wide range of Recurrence Scores. Gene expression levels for the proliferation genes were generally lower for the DCIS components in both studies (Supplementary Figure 1, available online). These results indicated that low Recurrence Score biology was not uniformly observed for DCIS and suggested that more aggressive biology for invasive breast carcinoma identified by Recurrence Score genes might also be present in DCIS. Other reports have also shown that proliferation gene and protein expression levels were associated with local recurrence risk for DCIS (28–30).

Although selection of the Recurrence Score algorithm without modification was considered for DCIS, the algorithm was modified before the clinical validation study to have a score that would be predictive of local recurrence risk regardless of adjuvant tamoxifen use because tamoxifen use for DCIS is variable. Selection of the final genes and DCIS Score algorithm used published results for the 21 individual genes from invasive breast carcinoma studies (23–25). These studies showed that proliferation gene group score, *PR* (progesterone receptor), and *GSTM1* predicted distant recurrence and breast cancer mortality in both tamoxifen-treated and -untreated patients. Other genes, including *ER* (estrogen receptor), were primarily predictive of hormonal therapy benefit. The seven genes that were purely predictive of recurrence risk plus five reference genes were selected for the DCIS Score (Figure 1). In contrast with the Recurrence Score, the DCIS Score algorithm does not threshold the proliferation group score. Scaling of the DCIS Score and selection of the specific cutpoints for the three risk groups were based primarily on the distribution of scores in the DCIS cohort from Marin General Hospital.

### Calculation of the DCIS Score

The calculation algorithm for the DCIS Score is as follows. The DCIS Score is scaled from zero to 100 and is derived from the reference normalized gene expression measurements in four prespecified steps. First, expression for each of the seven cancer-related genes is normalized relative to the expression of the five reference genes (*ACTB*, *GAPDH*, *RPLPO*, *GUS*, and *TFRC*). Reference-normalized expression measurements range from two to 15, with a one-unit increase reflecting approximately a doubling of RNA. Second, the proliferation group score is calculated as the average of the five proliferation genes as follows: proliferation group score = (*Ki67* + *STK15* + *Survivin* + *CCNB1* + *MYBL2*)/5. Third, the unscaled DCIS Score$_{\mu}$ is calculated as:

$$\text{DCIS Score}_{\mu} = +0.31 \times \text{proliferation group score} - 0.08 \times PR - 0.09 \times GSTM1.$$

A plus sign indicates that increased expression is associated with an increased risk of an ipsilateral breast event (IBE), and a minus sign indicates that increased expression is associated with a decreased risk of an IBE. Fourth, the DCIS Score is rescaled from the unscaled score as follows: DCIS Score = (66.7 × DCIS Score$_{\mu}$) + 10.0. If the DCIS Score is less than zero, then the DCIS Score equals zero. If the DCIS Score is greater than 100, then the DCIS Score equals 100. Three risk categories were prespecified: 1) low risk (DCIS Score < 39); 2) intermediate risk (DCIS Score = 39–54); and 3) high risk (DCIS Score ≥ 55).

### Validation of the DCIS Score

The ECOG E5194 study was chosen as an independent study to validate the DCIS Score. ECOG E5194 was a nonrandomized, prospective, multicenter study that was designed to evaluate treatment using surgical excision without radiation for selected women with DCIS (18). Patients were enrolled on the parent study through ECOG or the North Central Cancer Treatment Group.

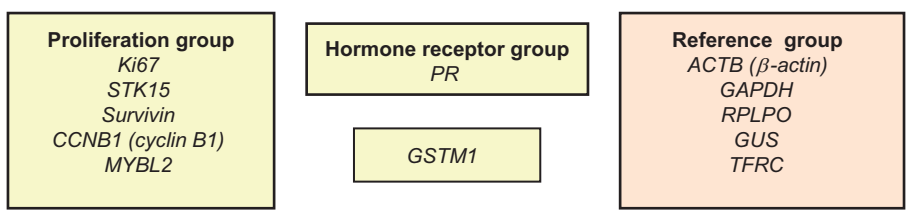


**Figure 1.** Panel of 12 genes included in the DCIS Score. Seven cancer-related genes: *Ki67* = MKI67; *STK15* = aurora kinase A; *survivin* = BIRC5; *CCNB1* = cyclin B1; *MYBL2* = v-myb myeloblastosis viral oncogene homolog (avian)-like 2; *PR* = progesterone receptor; and *GSTM1* = glutathione S-transferase M1. Five reference genes: *ACTB* = beta-actin; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase; *RPLPO* = large ribosomal protein; *GUS* = beta-glucuronidase; and *TFRC* = transferrin receptor.

Eligible patients had either 1) low- or intermediate-grade DCIS with tumor size less than or equal to 2.5 cm (cohort 1) or 2) high-grade DCIS with tumor size less than or equal to 1.0 cm (cohort 2). Protocol specifications included a minimum negative margin width of at least 3 mm or no tumor on reexcision. There were 670 eligible patients enrolled between April 1997 and October 2002. Adjuvant tamoxifen was optional beginning in May 2000, and was not randomized.

All patients provided written informed consent for future research in the parent protocol consent, which was approved by the institutional review board at each participating center. Separate institutional review board approval was also obtained for this study. The clinical trial registration number is NCT01132560 (31).

### Gene Assay Methods

The Onco*type* DX breast cancer assay was performed for all available specimens from ECOG E5194 using standardized methods (23,32–35). Using individual expression data from the 21 genes, two separate scores for each DCIS tumor were calculated using predefined algorithms: 1) the 12-gene DCIS Score and 2) the 21-gene Recurrence Score.

A small difference was identified in one reagent calibration factor for one of the proliferation genes after the abstract presentations (36,37). The corrected factor was used for the results reported here.

### Pathology

For this validation study, two expert breast pathologists (F.L. Baehner and S. Badve) performed concurrent central pathology review using the College of American Pathologists DCIS guidelines (30,37,38). Tumor size was defined as the largest microscopic dimension, including discontinuous areas (18,37,38). In the parent study, central pathology review was performed by an expert breast pathologist (D.L. Page) using different criteria (39). Local pathology assessment (using unspecified criteria) on entry into the parent study was also recorded.

### Statistical Analysis and Study Endpoints

The DCIS Score, primary and secondary study objectives, analytic methodology, and statistical plan were documented and finalized before the study was conducted. The primary objective was to determine whether the continuous DCIS Score was statistically significantly associated with the risk of an IBE (defined as local recurrence of DCIS or invasive carcinoma in the ipsilateral breast) using a Cox proportional hazards regression model. Tamoxifen use was included as a time-dependent variable because some patients began tamoxifen during follow-up. Statistical significance was based on a likelihood ratio test of $P$ less than .05.

Contingent on statistical significance of the DCIS Score, the second primary objective was to determine whether the Recurrence Score was statistically significantly associated with IBE risk for patients with hormone receptor–positive DCIS on reverse-transcription polymerase chain reaction. Diagnostics were performed to determine whether the assumptions of linearity and proportional hazards were met. For the Cox proportional hazards models with the continuous DCIS Score as a main effect, diagnostics based on Martingale and Schoenfeld residuals were conducted to evaluate the assumptions of functional form and proportional hazards, and these diagnostics supported the main effect models for evaluation of the DCIS Score as a continuous variable.

Because the results for the DCIS Score and the Recurrence Score were similar in the analyses with and without adjustment for tamoxifen, only results without adjustment are presented for secondary analyses.

Univariable and multivariable Cox models determined which clinical and pathologic variables were statistically significantly associated with IBE risk and whether the DCIS Score was statistically significantly associated with IBE risk with adjustment for these variables. Kaplan–Meier estimates of IBE risk through 10 years were calculated. A Cox model with the continuous DCIS Score as the main effect was used to estimate 10-year IBE risk as a function of the DCIS Score, based on the empirical cumulative hazard function, and 95% confidence intervals (CIs) were calculated using the log–log transform approach. Exploratory subgroup analyses were conducted based on clinical and pathologic characteristics, including Kaplan–Meier estimates of IBE risk according to DCIS Score group and Cox models for IBE risk as a function of the continuous DCIS Score.

Statistical tests were two-sided. Analyses were conducted jointly by ECOG and Genomic Health, Inc biostatisticians (R. Gray, S. M. Butler, and C. Yoshizawa). Median follow-up was 8.8 years (range = 0.2–13.2 years).

## Results

### Characteristics of the Patients for the Validation Study

There were 327 patients (49% of the parent study) with sufficient tissue for RNA extraction and multigene expression analysis (Figure 2). Patient characteristics are shown in Table 1. There were no differences between the characteristics of the 327 patients included in this study and the 343 patients not included, except for a small difference in tumor size.

An IBE developed in 46 patients (n = 26 DCIS only and n = 20 invasive carcinoma). The 10-year IBE rates were 14.6% for cohort 1 (low- or intermediate-grade DCIS, tumor size ≤ 2.5 cm) and 19.0% for cohort 2 (high-grade DCIS, tumor size ≤ 1 cm) (Supplementary Figure 2, available online). There was no statistically significant relationship between IBE risk and grade as determined by local pathology or expert central pathology review (Supplementary Figure 3, available online).

### Analysis of the DCIS Score

In the prespecified primary analysis, the DCIS Score as a continuous variable was significantly associated with developing an IBE when adjusted for tamoxifen use (hazard ratio [HR] = 2.31, 95% CI = 1.15 to 4.49; $P$ = .02) (Table 2). Without adjustment for tamoxifen use, the hazard ratio was essentially unchanged (HR = 2.38, 95% CI = 1.19 to 4.60; $P$ = .01) (Supplementary Table 3, available online). For invasive IBE, the hazard ratio was 3.68 (95% CI = 1.34 to 9.62; $P$ = .01).

Univariable analyses showed that age, menopausal status, and tumor size were statistically significant, but other variables were not (Table 3; Supplementary Table 4, available online). In multivariable analyses, factors statistically significantly associated with developing an IBE were DCIS Score, tumor size, and menopausal status (all $P \leq .02$) (Table 4). The hazard ratio for the DCIS

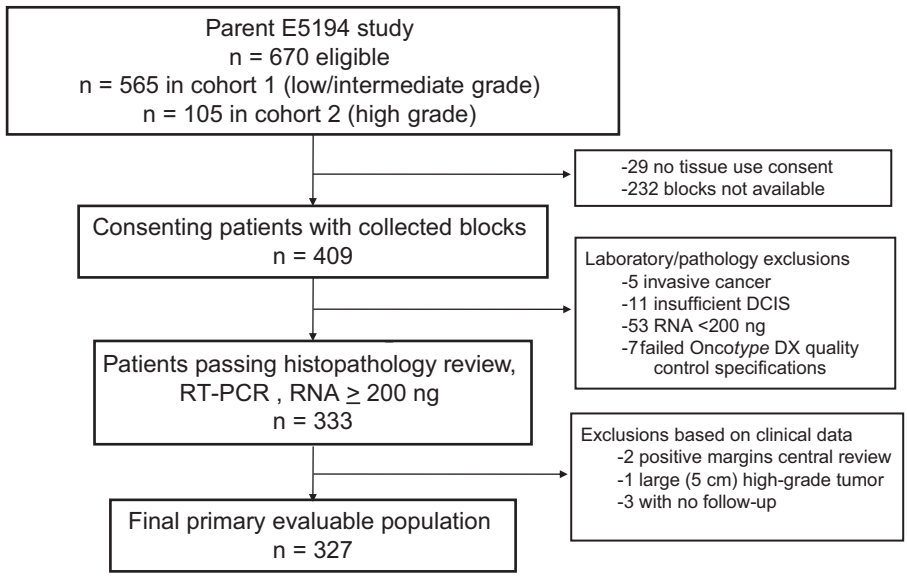


**Figure 2.** Consolidated Standards of Reporting Trials flow diagram for study numbers. DCIS = ductal carcinoma in situ; RT-PCR = reverse-transcription polymerase chain reaction.

Score was essentially unchanged when adjusted for tumor size and menopausal status, indicating that the DCIS Score provided independent information for IBE risk. The hazard ratios for the DCIS Score were also not decreased when adjusted for other clinical or pathologic variables (data not shown).

Using the DCIS Score for the three prespecified risk groups, the 10-year rates of developing an IBE were 10.6%, 26.7%, and 25.9% for the low-, intermediate-, and high- risk groups, respectively (log rank $P = .006$) (Figure 3). The corresponding 10-year rates of developing an invasive IBE were 3.7%, 12.3%, and 19.2%, respectively (log rank $P = .003$). The 10-year estimated risk of an IBE or invasive IBE increased continuously as the DCIS Score increased.

In contrast with the DCIS Score, the Recurrence Score was not statistically significantly associated with developing an IBE (Supplementary Figure 4, available online). No statistically significant association was seen for developing a contralateral breast cancer for either the DCIS Score or the Recurrence Score (Supplementary Figure 5, available online).

### Exploratory Analyses and Other Endpoints

Although underpowered, exploratory analyses were performed for several clinically relevant subgroups (Figure 4; Supplementary Figure 6, available online). These subgroup analyses generally showed that the associations of the DCIS Score with IBE risk had similar trends and were directionally consistent with the overall group of patients, although with wide confidence intervals. The DCIS Score association with IBE risk was consistent with or without tamoxifen treatment. Scatter plots showed a wide range of DCIS Scores across subgroups (Supplementary Figure 7, available online).

The proliferation group scores showed a wide range of values (Supplementary Figure 8, available online), which resulted in considerable impact for calculating the DCIS Score. Of the hormone receptor–positive DCIS tumors, 96.9% (n = 310 of 320) had a proliferation group score below the threshold value of 6.5, which resulted in a negligible contribution for calculating the Recurrence Score. In the analysis of expression levels for the 21 individual genes in the Oncotype DX assay, the proliferation genes were strongly associated with IBE risk and invasive IBE risk (Supplementary Figure 9, available online).

## Discusssion

This prospective–retrospective study has demonstrated that the multigene DCIS Score quantified the 10-year risk of local recurrence and invasive local recurrence for selected patients with DCIS of the breast treated with surgical excision without radiation. Whether used as a continuous or categorical variable, the DCIS Score predicted the 10-year risk of local recurrence and provided information for recurrence risk that was independent of traditional clinical and pathologic factors.

The study population used was from the ECOG E5194 study, which was a prospective trial of patients selected for treatment with surgical excision without radiation based on low-risk features (18). Despite favorable selection criteria, the 10-year risks of IBE for the two cohorts defined from the parent study were 14.6% and 19.0%, respectively, indicating that traditional clinical and pathologic criteria alone were insufficient to define a low-risk population (Supplementary Figure 2, available online).

The relative risk of developing an IBE as estimated by the DCIS Score was both statistically significant and clinically meaningful (Figure 3). The 10-year risk can be expressed as a continuous function of the DCIS Score with associated confidence intervals, where the estimates were more precise for lower-risk scores. The risk of an IBE was more than twofold higher for the 30% of patients with an intermediate or high DCIS Score compared with the 70% of patients with a low DCIS Score.

As most women with newly diagnosed DCIS are eligible for breast conservation surgery, clinical decision making frequently centers on whether to add radiation or not and, by extension, on

**Table 1.** Patient, tumor, and treatment characteristics for the 327 patients from Eastern Cooperative Oncology Group E5194 included in this validation study and for the 343 patients not included in this study*

| Characteristic | Included, No. (%) | Not included, No. (%) |
|---|---|---|
| Overall | 327 (100) | 343 (100) |
| Cohort arm from the parent study† | | |
| Cohort 1 | 273 (83.5) | 292 (85.1) |
| Cohort 2 | 54 (16.5) | 51 (14.9) |
| Age, years | | |
| ≤39 | 8 (2.4) | 9 (2.6) |
| 40–49 | 58 (17.7) | 57 (16.6) |
| 50–59 | 86 (26.3) | 112 (32.7) |
| ≥60 | 175 (53.5) | 165 (48.1) |
| Menopausal status‡ | | |
| Premenopausal | 79 (24.2) | 83 (24.6) |
| Postmenopausal | 248 (75.8) | 254 (75.4) |
| Tumor size, mm | | |
| ≤5 | 104 (31.8) | 152 (44.3) |
| 6–10 | 156 (47.7) | 138 (40.2) |
| >10 | 67 (20.5) | 53 (15.5) |
| Tamoxifen use | | |
| Yes§ | 96 (29.4) | 105 (30.6) |
| No | 231 (70.6) | 238 (69.4) |
| Minimum negative margin width, mm | | |
| <1‖ | 5 (1.5) | 7 (2.0) |
| 1–2.9‖ | 5 (1.5) | 7 (2.0) |
| 3–4.9 | 103 (31.5) | 110 (32.1) |
| 5–9.9 | 144 (44.0) | 145 (42.3) |
| ≥10¶ | 70 (21.4) | 74 (21.6) |
| Central grade, this study | | |
| Low | 29 (8.9) | |
| Intermediate | 187 (57.2) | |
| High | 111 (33.9) | |
| DCIS pattern, this study | | |
| Solid | 116 (35.5) | |
| Cribriform | 174 (53.2) | |
| Micropapillary | 23 (7.0) | |
| Papillary | 14 (4.3) | |
| Comedo necrosis, this study | | |
| None | 213 (65.1) | |
| 1%–30% | 50 (15.3) | |
| >30% | 64 (19.6) | |
| Estrogen receptor status by RT-PCR | | |
| Negative | 9 (2.8) | |
| Positive | 318 (97.2) | |
| Progesterone receptor status by RT-PCR | | |
| Negative | 28 (8.6) | |
| Positive | 299 (91.4) | |
| Hormone receptor status by RT-PCR# | | |
| Negative | 7 (2.1) | |
| Positive | 320 (97.9) | |
| *HER2* status by RT-PCR | | |
| Negative | 280 (85.6) | |
| Equivocal | 22 (6.7) | |
| Positive | 25 (7.6) | |

* For comparison of the two groups of included patients vs not included patients, only tumor size was statistically significantly different ($P = .003$), whereas all $P$ values were greater than or equal to 0.33 for the remaining characteristics, based on $\chi^2$ tests. All statistical tests were two-sided. DCIS = ductal carcinoma in situ; *HER2* = human epidermal growth factor receptor 2; RT-PCR = reverse-transcription polymerase chain reaction.

† Cohort 1: low or intermediate grade, tumor size less than or equal to 2.5 cm; Cohort 2: high grade, tumor size less than or equal to 1.0 cm.

‡ Excludes cases with missing information.

§ This study included three patients with tamoxifen use after development of a contralateral breast cancer, but before an ipsilateral breast event.

‖ Ten patients included in this study were found on central pathology review for the parent study to have a surgical margin less than 3 mm but no tumor at the margins of resection. These 10 cases were included in this study to be consistent with the criteria for inclusion in the analysis of the parent study analysis as reported by Hughes et al. (18).

¶ Includes cases with no tumor on re-excision.

# Hormone receptor positive was defined as estrogen receptor positive and/or progesterone receptor positive. Hormone receptor negative was defined as both estrogen receptor negative and progesterone receptor negative.

**Table 2.** Primary analysis: Cox proportional hazards regression analysis of the DCIS Score and the Recurrence Score as predictors of risk of an ipsilateral breast event with adjustment for tamoxifen use

| Variables | No. of Patients | Hazard ratio* (95% CI) | *P*† |
|---|---|---|---|
| DCIS Score, prespecified primary analysis | 327 | | |
| DCIS Score | | 2.31 (1.15 to 4.49) | .02 |
| Tamoxifen use | | 0.56 (0.24 to 1.15) | .12 |
| Recurrence Score, prespecified conditional analysis‡ | 320§ | | |
| Recurrence Score | | 0.75 (0.16 to 2.79) | .69 |
| Tamoxifen use | | 0.56 (0.24 to 1.16) | .12 |

* Hazard ratios for the DCIS Score and for the Recurrence Score are for a prespecified 50-point difference, which results in a hazard ratio that is comparable with the hazard ratio for the high DCIS Score group (or intermediate DCIS Score group) compared with the low DCIS Score group (Supplementary Table 3, available online). CI, confidence interval.

† Likelihood ratio test based on Cox proportional hazards regression. All statistical tests were two-sided.

‡ Prespecified conditional analysis based on obtaining a statistically significant *P* value for the DCIS Score in the prespecified primary analysis.

§ The Recurrence Score analysis excludes seven patients with hormone receptor–negative DCIS tumors.

**Table 3.** Univariable Cox proportional hazards models for the risk of an ipsilateral breast event for selected clinical and pathologic variables*

| Variables | Hazard ratio† (95% CI) | *P* ‡ |
|---|---|---|
| Age§ | 0.78 (0.60 to 1.00) | .05 |
| Menopausal status | | .03 |
| Premenopausal | 1.00 (referent) | |
| Postmenopausal | 0.51 (0.29 to 0.95) | |
| Tumor size§ | 1.51 (1.12 to 2.00) | .009 |
| Tumor size, mm | | .31 |
| ≤5 | 1.00 (referent) | |
| 6–10 | 1.16 (0.58 to 2.44) | |
| >10 | 1.81 (0.82 to 4.03) | |
| Tamoxifen use | | .09 |
| No | 1.00 (referent) | |
| Yes | 0.54 (0.23 to 1.09) | |
| Minimum negative margin width, mm | | .47 |
| <5 | 1.00 (referent) | |
| 5–9 | 0.75 (0.38 to 1.50) | |
| ≥10‖ | 1.18 (0.56 to 2.42) | |
| Cohort arm from the parent study¶ | | .83 |
| Cohort 1 | 1.00 (referent) | |
| Cohort 2 | 1.09 (0.49 to 2.16) | |
| Grade, this study | | .69 |
| Low | 1.00 (referent) | |
| Intermediate | 1.44 (0.51 to 6.01) | |
| High | 1.14 (0.38 to 4.95) | |
| Comedo necrosis, this study | | .47 |
| None | 1.00 (referent) | |
| 1%–30% | 0.80 (0.30 to 1.81) | |
| >30% | 1.42 (0.69 to 2.72) | |
| Van Nuys Prognostic Index# | | .90 |
| Low risk | 1.00 (referent) | |
| Intermediate risk | 1.04 (0.57 to 1.86) | |

* See Supplementary Table 4 (available online) for complete list of all clinical and pathologic variables evaluated. CI = confidence interval.

† The hazard ratio for age as a continuous variable is for a 10-year difference, and the hazard ratio for tumor size as a continuous variable is for a 5-mm difference.

‡ Likelihood ratio test based on Cox proportional hazards regression. All statistical tests were two-sided.

§ Evaluated as a continuous variable.

‖ Includes cases with no tumor on reexcision.

¶ Cohort 1: low or intermediate grade, tumor size less than or equal to 2.5 cm; Cohort 2: high grade, tumor size less than or equal to 1.0 cm

# Includes 203 low-risk (score 4–6) case subjects and 124 intermediate-risk (score 7–9) case subjects as determined using the Van Nuys Prognostic Index (20).

estimating the baseline risk of IBE and invasive IBE after surgical excision without radiation. For patients at low risk, national guidelines include the option for surgical excision alone as acceptable treatment, although the definition of low risk is not well defined (40,41). The DCIS Score can aid clinical decision making by identifying those patients with a lower DCIS Score for whom surgical

**Table 4.** Multivariable Cox proportional hazards models for the risk of an ipsilateral breast event

| Analyses and variables | Hazard ratio (95% CI)* | *P*† |
|---|---|---|
| Multivariable analysis of significant clinical and pathologic factors, excluding the DCIS Score | | |
| Menopausal status | | .02 |
| Premenopausal | 1.00 (referent) | |
| Postmenopausal | 0.49 (0.27 to 0.90) | |
| Tumor size‡ | 1.54 (1.14 to 2.02) | .006 |
| Multivariable analysis of significant clinical and pathologic factors, including the DCIS Score | | |
| Menopausal status | | .02 |
| Premenopausal | 1.00 (referent) | |
| Postmenopausal | 0.49 (0.27 to 0.90) | |
| Tumor size‡ | 1.52 (1.11 to 2.01) | .01 |
| DCIS Score‡ | 2.37 (1.14 to 4.76) | .02 |

* The hazard ratio for the DCIS Score is for a 50-point difference. Hazard ratios for tumor size are for a 5-mm difference. CI = confidence interval.

† Likelihood ratio test based on Cox proportional hazards regression. All statistical tests were two-sided.

‡ Evaluated as a continuous variable.

excision alone may be adequate and those patients with a higher DCIS Score for whom adding treatment after surgical excision should be considered.

Pathologic grade commonly influences clinical management decisions for DCIS. This analysis included three different assessments of grade, and there was no statistically significant association (Supplementary Figure 3, available online). The lack of association between grade and IBE risk has previously been reported and may be due to differing grading systems, interobserver variability, heterogeneity of grade within DCIS, selection of small (≤1.0 cm) high-grade DCIS in this study, or true lack of association between grade and IBE risk (30,37,42,43).

In contrast with the DCIS Score, the Recurrence Score was not associated with IBE risk (Supplementary Figure 4, available online), indicating the importance of both gene selection and calculation algorithm for DCIS. Calculation of the DCIS Score includes the full range of the proliferation gene group score, whereas calculation of the Recurrence Score includes a thresholded proliferation score (Supplementary Figures 1 and 8, available online). Thresholding the proliferation group score for calculating the Recurrence Score explains in large part its lack of association with IBE risk and highlights the fact that expression of the proliferation genes for DCIS is different from expression of the proliferation genes for invasive breast carcinoma.

Randomized clinical trials have demonstrated that adding radiation after surgical excision is associated with an approximately 50% reduction in local recurrence and invasive local recurrence (5–14). In the Early Breast Cancer Trialists' Collaborative Group meta-analysis of randomized trials, 10-year local recurrence was reduced from 28.1% after lumpectomy alone to 12.9% with radiotherapy ($P < .00001$) (12). However, randomized trials have not shown a difference in distant metastases or survival.

Two randomized clinical trials have shown that adding adjuvant tamoxifen statistically significantly reduced the risk of all breast cancer events (combined ipsilateral plus contralateral) (6,7,10,44,45). Only 29.4% of patients in this study were treated with tamoxifen (Table 1), which suggests that slightly lower event rates would be expected if tamoxifen were used.

This study has several notable strengths. This study used a rigorous prospective–retrospective methodology, including prespecified DCIS Score, study population, analytical and statistical methodologies, study objectives, and central expert pathology review using both older and contemporary grading classifications. The study population was representative of the parent study and thus is relevant to current practice for those patients with DCIS meeting the enrollment criteria for the ECOG E5194 study.

This study has several potential limitations. First, development of the DCIS Score relied in part on studies of invasive breast carcinoma for gene selection and algorithm development. This strategy was necessary because of lack of DCIS studies with formalin-fixed paraffin-embedded tissue and documented negative surgical margins to allow for adequately powered discovery of genes associated with local recurrence. Second, tamoxifen was not randomized in the ECOG E5194 parent study, and therefore the study results cannot be interpreted as evidence for or against the benefit of tamoxifen. However, tamoxifen was not a confounder on multivariable analysis and, therefore, does not affect the conclusion regarding the association of the DCIS Score with IBE risk. Third, sample sizes for subgroups were limited. Nonetheless, subgroup analyses were consistent with the findings for the overall group of patients. Finally, there were relatively few patients with DCIS tumors that were hormone receptor negative or *HER2* positive. Additional confirmatory studies, including patient populations beyond the ECOG E5194 enrollment criteria, are warranted.

In summary, the DCIS Score predicts the risks of local recurrence and invasive local recurrence and provides information that complements traditional clinical and pathologic factors for this study population of women with DCIS treated with surgical excision without radiation. The differences in the risks of developing local recurrence and invasive local recurrence between patients with a lower DCIS Score and a higher DCIS Score were statistically significant and clinically meaningful. The DCIS Score provides a new clinical tool to quantify local recurrence risk and to guide individualized selection of treatment after surgical excision for women with newly diagnosed DCIS who meet the ECOG E5194 criteria.

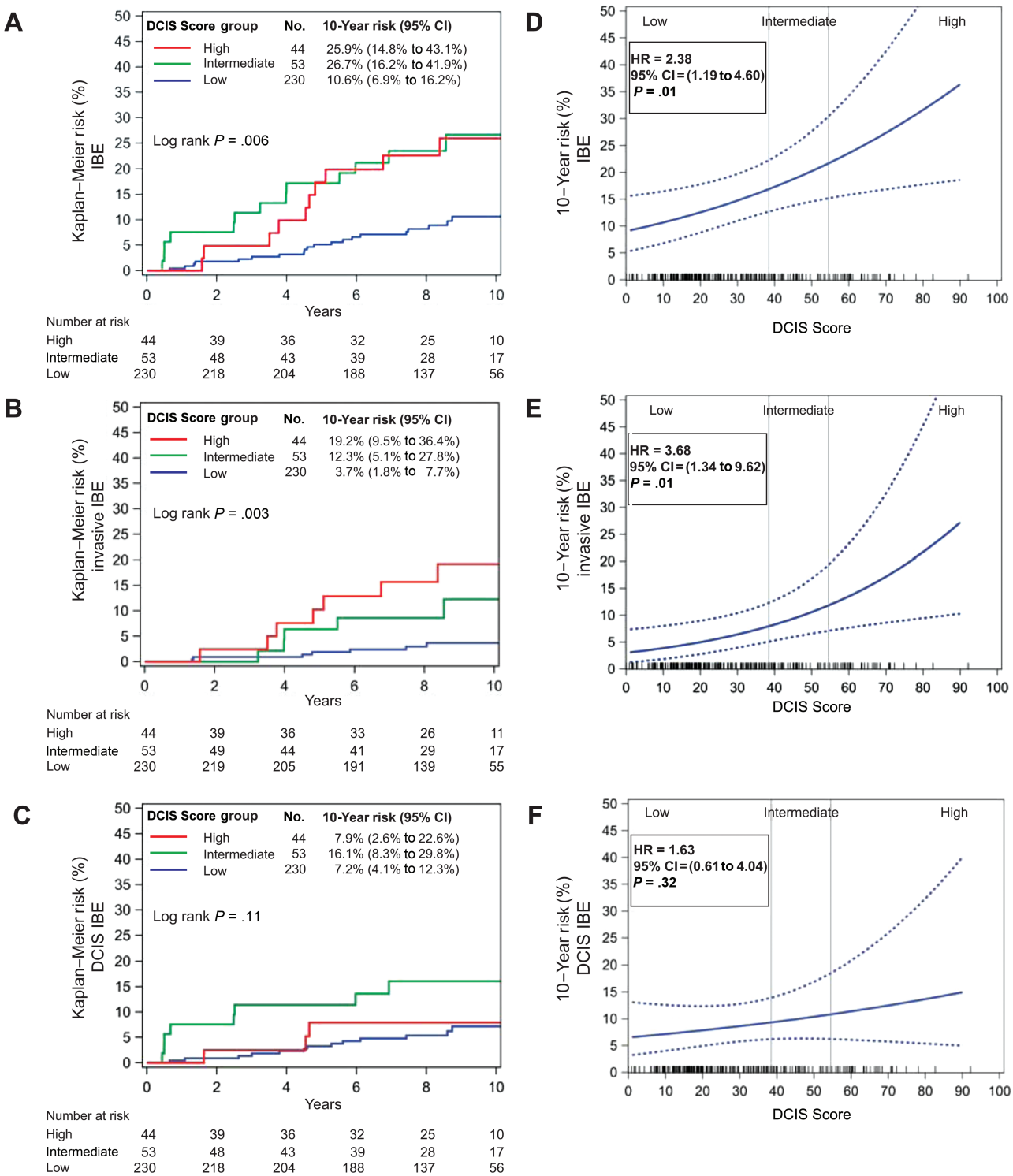


**Figure 3.** Kaplan–Meier plots and 10-year risk estimates with 95% confidence intervals (CIs) for developing an ipsilateral breast event (IBE), an invasive IBE, and a ductal carcinoma in situ (DCIS) IBE. The number of patients at risk are included below each plot for each prespecified risk group, based on the DCIS Score of low (<39), intermediate (39–54), and high (≥55). **A**) Probability of developing an IBE based on the DCIS Score according to the three prespecified risk groups. **B**) Probability of developing an invasive IBE based on the DCIS Score according to the three prespecified risk groups. **C**) Probability of developing a DCIS IBE (censored if an invasive IBE occurred) based on the DCIS Score according to the three prespecified risk groups. **D**) Estimated 10-year risk of developing an IBE as a continuous function using the DCIS Score based on a Cox proportional hazards model, including 95% confidence intervals demonstrating the level of precision in the estimates. More precise estimates are seen for lower values and lower risk levels because of the greater number of observations, as indicated in the rug plot along the x-axis. The hazard ratios are presented for a 50-point difference in the DCIS Score. **E**) Estimated 10-year risk of developing an invasive IBE as a continuous function using the DCIS Score based on a Cox proportional hazards model, including 95% confidence intervals. **F**) Estimated 10-year risk of developing a DCIS IBE as a continuous function using the DCIS Score based on a Cox proportional hazards model, including 95% confidence intervals.

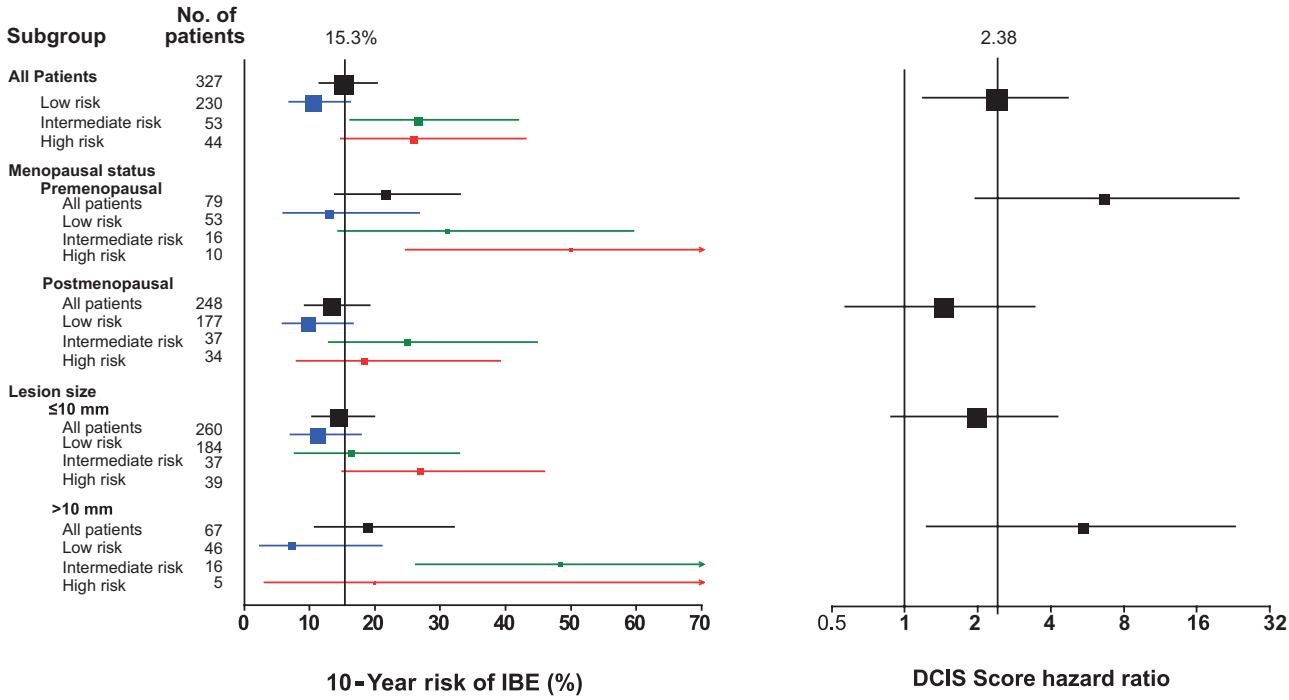


**Figure 4.** Subgroup analyses for variables found to be statistically significantly associated with an ipsilateral breast event (IBE) in the multivariable models. The **left side** of the figure shows the Kaplan–Meier estimates of the 10-year risk of any IBE (with 95% confidence interval) according to the DCIS Score prespecified risk groups. **Blue boxes** are estimates for the low DCIS Score risk group and are generally to the left of the overall IBE risk estimate of 15.3%. **Green boxes** are estimates for the intermediate DCIS Score risk group. **Red boxes** are estimates for the high DCIS Score risk group, and are generally to the right of the overall IBE risk estimate. The **box size** is proportional to the number of patients. The **right side** of the figure shows the hazard ratios for IBE risk, with 95% confidence intervals. The hazard ratios are calculated for a 50-point difference in the continuous DCIS Score. For each subgroup, the 95% confidence intervals overlap the hazard ratio of 2.38 for the overall group of 327 patients.

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**Affiliations of authors:** Department of Radiation Oncology, Albert Einstein Medical Center, Philadelphia, PA (LJS); Dana-Farber Cancer Institute, Boston, MA (RG); Eastern Cooperative Oncology Group Coordinating Center, Boston, MA (RG); Genomic Health, Inc, Redwood City, CA (FLB, SMB, CY, DBC, SS); Department of Pathology, University of California–San Francisco, San Francisco, CA (FLB); North Georgia Radiation Therapy, The Hope Center, Cartersville, GA (LLH); Department of Pathology, Vanderbilt University, Nashville, TN (DLP); Department of Medical Oncology (GWS) and Department of Pathology (SB), Indiana University, Indianapolis, IN; University of Pittsburgh Cancer Institute, Pittsburgh, PA (NED); Mayo Clinic, Rochester, MN (JNI); Mayo Clinic, Jacksonville, FL (EAP); Department of Surgery, Emory University, Atlanta, GA (WCW); Department of Medical Oncology, Montefiore Medical Center, Albert Einstein College of Medicine, New York, NY (JAS).