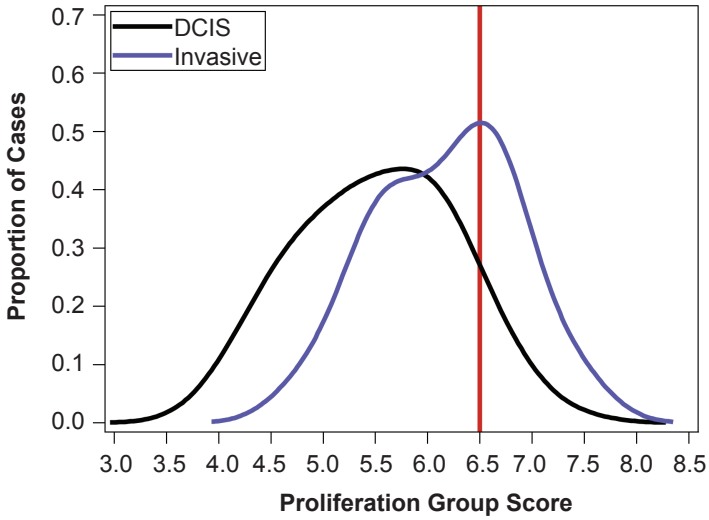
Supplementary Figure 1. Distribution of the proliferation group scores from pure ductal carcinoma in situ (DCIS) patient samples (n = 96) from Marin General Hospital (Greenbrae, CA) compared to invasive breast carcinoma patient samples (n = 74) used in the quality control process for the Onco*type* DX Breast Cancer Assay. The observed proliferation group scores were significantly less in pure DCIS compared to invasive breast carcinoma (t-test $P < 0.001$). A total of 88 (91.7%) of the DCIS samples and 46 (62.2%) of the invasive breast carcinoma samples showed proliferation group scores less than the proliferation threshold of 6.5 used in the Recurrence Score calculation. Kernel smoothed distributions are presented in which the area under the curve corresponds to the proportion of patients with a proliferation group score in the associated range.

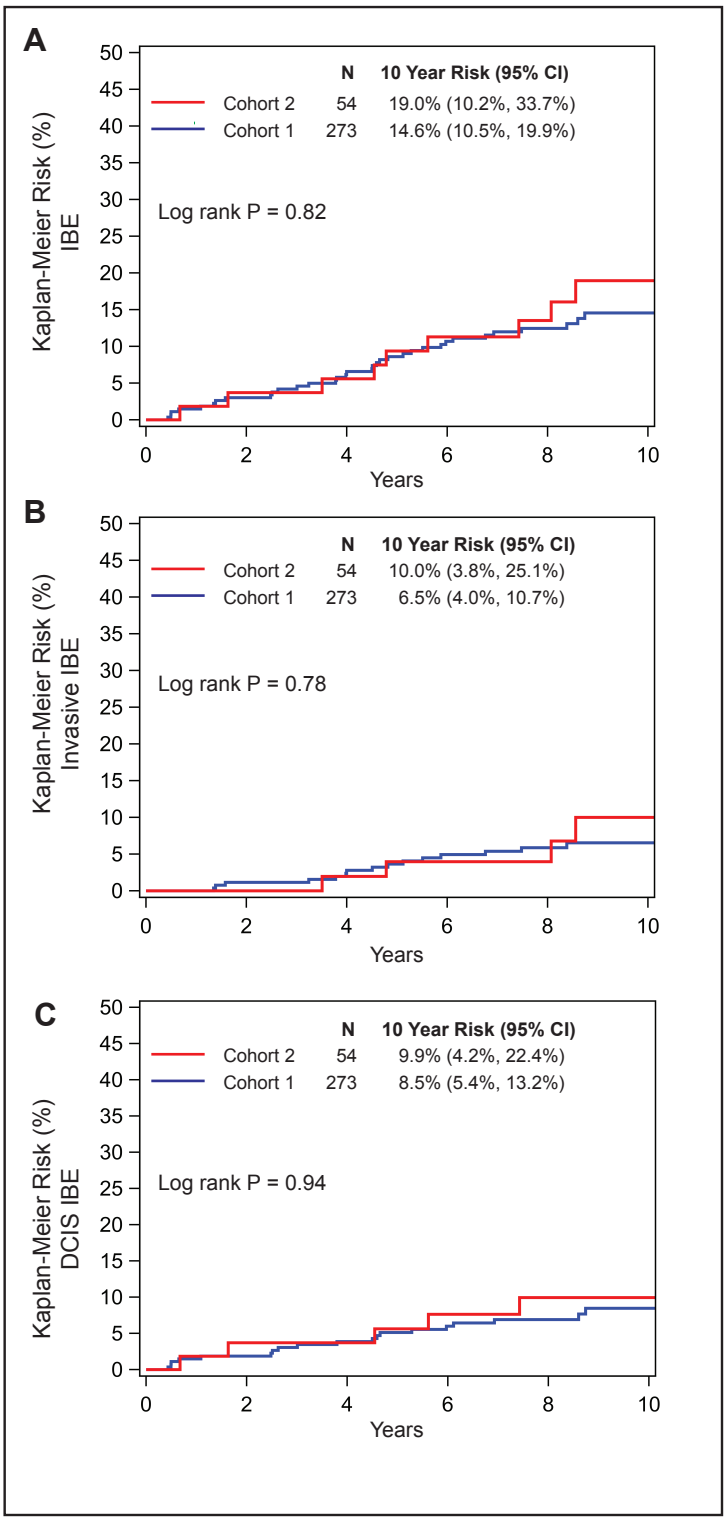

DCIS
Invasive
Proportion of Cases
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
Proliferation Group Score

Supplementary Figure 2. Kaplan-Meier plots and 10 year risk estimates with 95% confidence intervals (CI) for the probability of developing an ipsilateral breast event (IBE), invasive IBE, or DCIS IBE according to study cohort as determined at the time of entry into the parent study. For the parent study, Cohort 1 was defined as low or intermediate grade ductal carcinoma in situ (DCIS), tumor size ≤ 2.5 cm, and Cohort 2 was defined as high grade DCIS, tumor size ≤ 1.0 cm.

A. Probability of developing an IBE according to study cohort.

B. Probability of developing an invasive IBE according to study cohort.

C. Probability of developing a DCIS IBE according to study cohort.

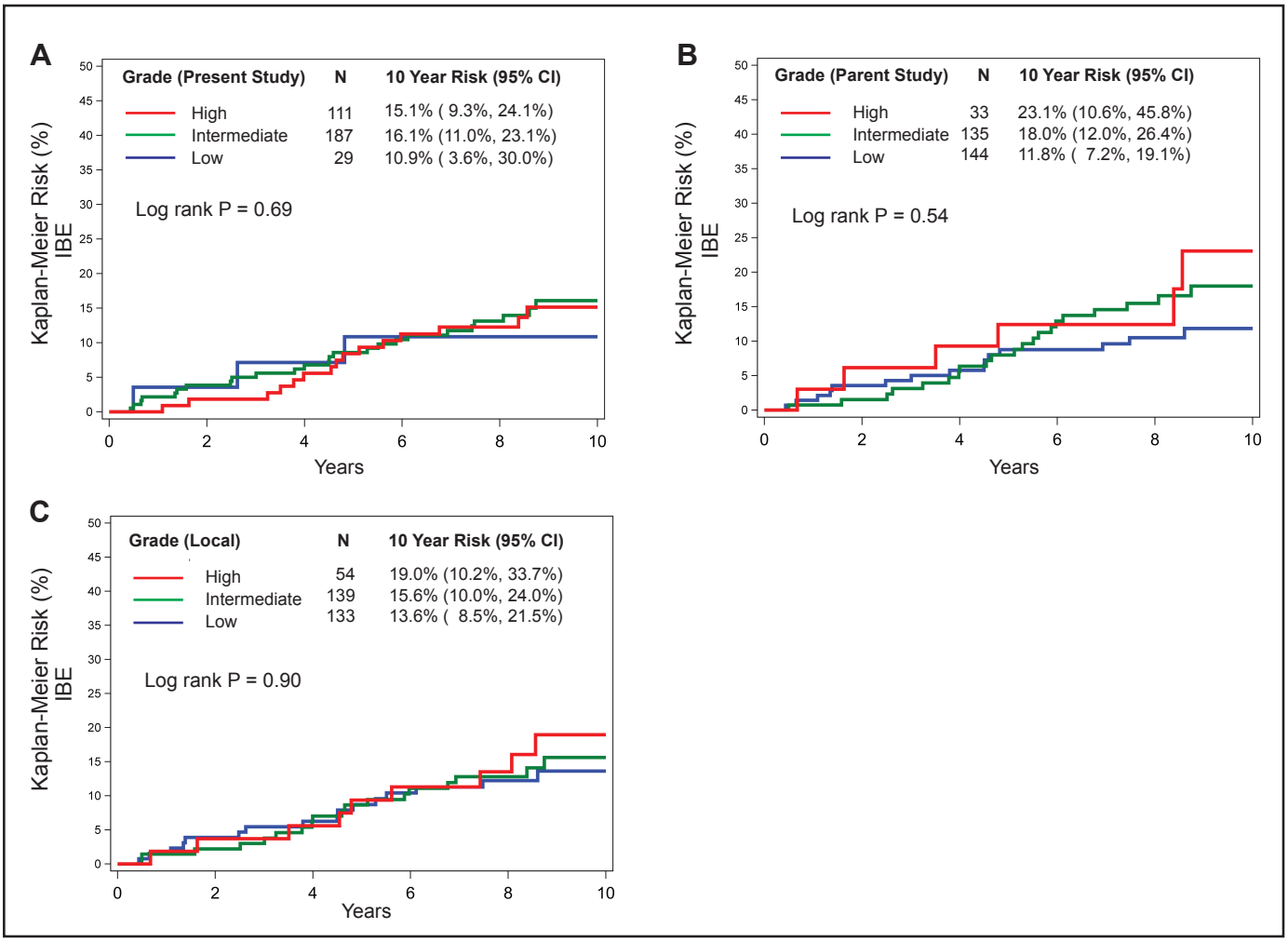

A
Kaplan-Meier Risk (%)
IBE
N
10 Year Risk (95% CI)
Cohort 2 54 19.0% (10.2%, 33.7%)
Cohort 1 273 14.6% (10.5%, 19.9%)
Log rank P = 0.82
Years
B
Kaplan-Meier Risk (%)
Invasive IBE
N
10 Year Risk (95% CI)
Cohort 2 54 10.0% (3.8%, 25.1%)
Cohort 1 273 6.5% (4.0%, 10.7%)
Log rank P = 0.78
Years
C
Kaplan-Meier Risk (%)
DCIS IBE
N
10 Year Risk (95% CI)
Cohort 2 54 9.9% (4.2%, 22.4%)
Cohort 1 273 8.5% (5.4%, 13.2%)
Log rank P = 0.94
Years

Supplementary Figure 3. Kaplan-Meier plots and 10 year estimates with 95% confidence intervals (CI) for the probability of developing an ipsilateral breast event (IBE) according to grade as determined by central pathology review for the present study, central pathology review of grade at the time of enrollment into the parent study, or local assessment at the time of enrollment into the parent study.

A. Probability of developing an IBE according to grade as determined by central pathology review of grade for the present study.

B. Probability of developing an IBE according to grade as determined by central pathology review of grade at the time of enrollment into the parent study.

C. Probability of developing an IBE according to grade as determined by local assessment at the time of enrollment into the parent study.

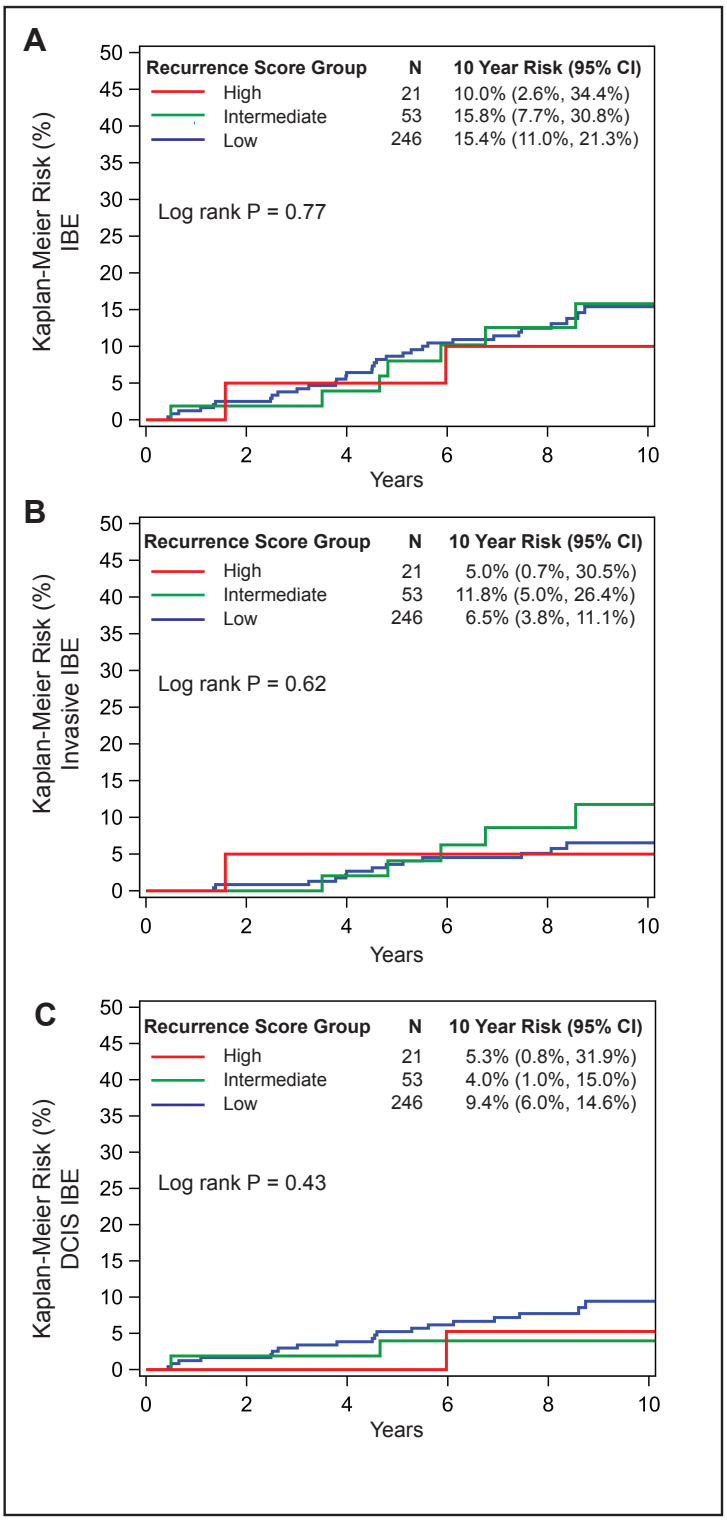

A
Grade (Present Study) N 10 Year Risk (95% CI)
High 111 15.1% (9.3%, 24.1%)
Intermediate 187 16.1% (11.0%, 23.1%)
Low 29 10.9% (3.6%, 30.0%)
Log rank P = 0.69
Kaplan-Meier Risk (%) IBE
Years
B
Grade (Parent Study) N 10 Year Risk (95% CI)
High 33 23.1% (10.6%, 45.8%)
Intermediate 135 18.0% (12.0%, 26.4%)
Low 144 11.8% (7.2%, 19.1%)
Log rank P = 0.54
Kaplan-Meier Risk (%) IBE
Years
C
Grade (Local) N 10 Year Risk (95% CI)
High 54 19.0% (10.2%, 33.7%)
Intermediate 139 15.6% (10.0%, 24.0%)
Low 133 13.6% (8.5%, 21.5%)
Log rank P = 0.90
Kaplan-Meier Risk (%) IBE
Years

Supplementary Figure 4. Kaplan-Meier plots and 10 year risk estimates with 95% confidence intervals (CI) for the probability of developing an ipsilateral breast event (IBE), invasive IBE, or DCIS IBE according to Recurrence Score risk group. Analyses according to Recurrence Score risk groups have been restricted to the 320 patients with hormone receptor positive ductal carcinoma in situ (DCIS) because the prespecified population for analysis of the Recurrence Score in the protocol for the present study was restricted to hormone receptor positive DCIS. Similar results were observed when all 327 patients were included (data not shown).

A. Probability of developing an ipsilateral breast event (IBE) according to Recurrence Score risk group.

B. Probability of developing an invasive IBE according to Recurrence Score risk group.

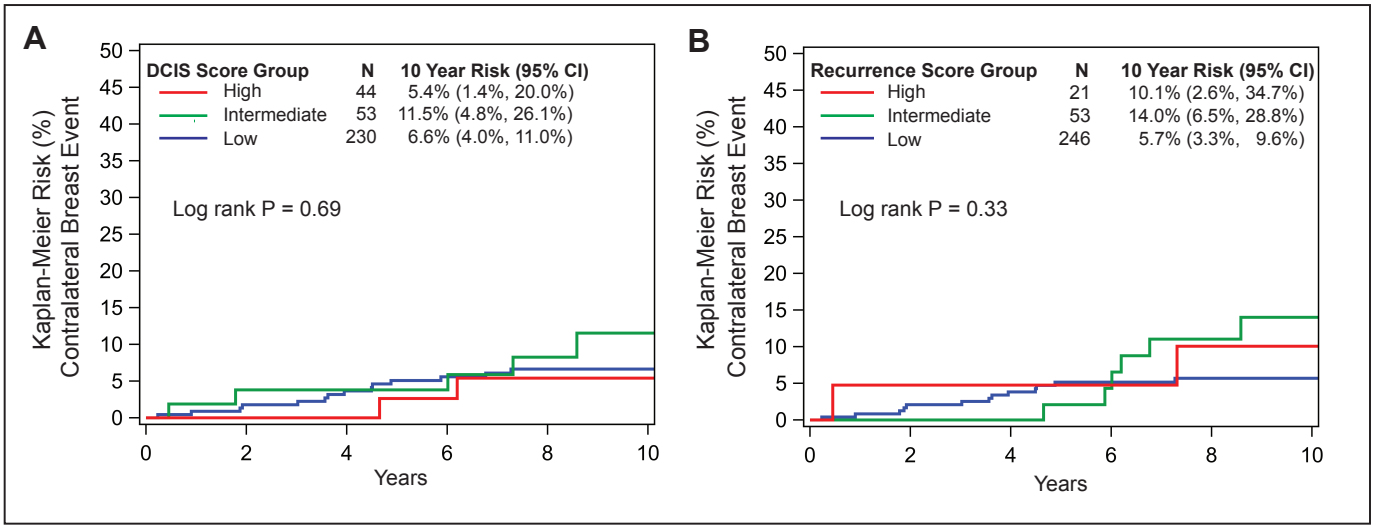
C. Probability of developing a DCIS IBE according to Recurrence Score risk group.


A
Kaplan-Meier Risk (%)
IBE
Recurrence Score Group
N
10 Year Risk (95% CI)
High
21
10.0% (2.6%, 34.4%)
Intermediate
53
15.8% (7.7%, 30.8%)
Low
246
15.4% (11.0%, 21.3%)
Log rank P = 0.77
Years
B
Kaplan-Meier Risk (%)
Invasive IBE
Recurrence Score Group
N
10 Year Risk (95% CI)
High
21
5.0% (0.7%, 30.5%)
Intermediate
53
11.8% (5.0%, 26.4%)
Low
246
6.5% (3.8%, 11.1%)
Log rank P = 0.62
Years
C
Kaplan-Meier Risk (%)
DCIS IBE
Recurrence Score Group
N
10 Year Risk (95% CI)
High
21
5.3% (0.8%, 31.9%)
Intermediate
53
4.0% (1.0%, 15.0%)
Low
246
9.4% (6.0%, 14.6%)
Log rank P = 0.43
Years

Supplementary Figure 5. Kaplan-Meier plots and 10 year risk estimates with 95% confidence intervals (CI) for the probability of developing a contralateral breast event (ductal carcinoma in situ [DCIS] only or invasive carcinoma) using the DCIS Score or the Recurrence Score.

A. Probability of developing a contralateral breast event using the DCIS Score.

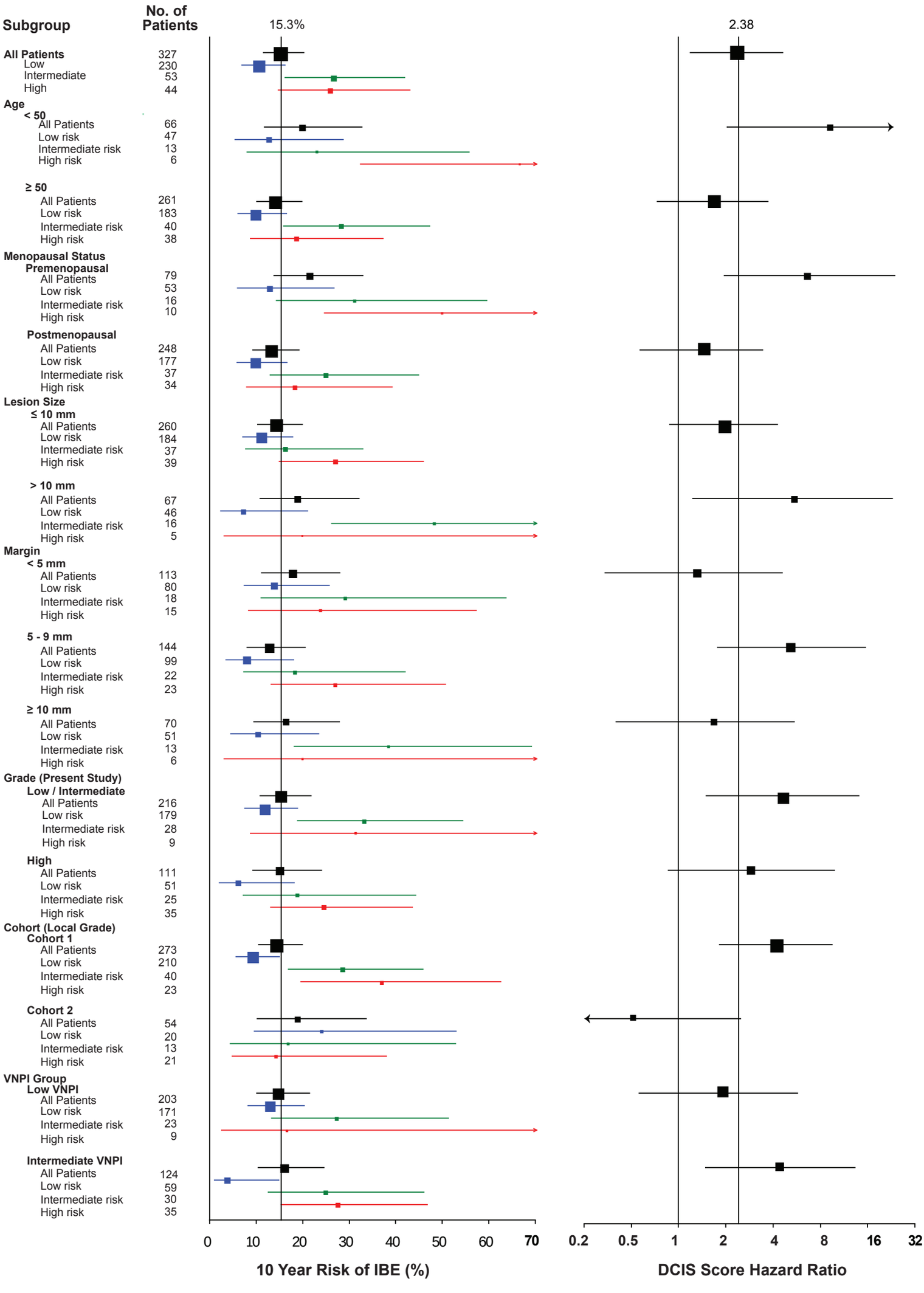
B. Probability of developing a contralateral breast event using the Recurrence Score.


A
Kaplan-Meier Risk (%)
Contralateral Breast Event
DCIS Score Group
N
10 Year Risk (95% CI)
High
44
5.4% (1.4%, 20.0%)
Intermediate
53
11.5% (4.8%, 26.1%)
Low
230
6.6% (4.0%, 11.0%)
Log rank P = 0.69
Years
B
Kaplan-Meier Risk (%)
Contralateral Breast Event
Recurrence Score Group
N
10 Year Risk (95% CI)
High
21
10.1% (2.6%, 34.7%)
Intermediate
53
14.0% (6.5%, 28.8%)
Low
246
5.7% (3.3%, 9.6%)
Log rank P = 0.33
Years

Supplementary Figure 6. Subgroup analyses of the DCIS Score as a predictor of risk of an ipsilateral breast event (IBE) risk for all clinical and pathologic subgroups examined. The left side of the figure shows Kaplan-Meier estimates of the 10-year risk of any IBE (with 95% confidence intervals) 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 based on Cox proportional hazards models. For each subgroup, the 95% confidence intervals overlap the hazard ratio of 2.38 for the overall group of 327 patients.

Abbreviation: VNPI = Van Nuys Prognostic Index (11).


Subgroup
No. of Patients
15.3%
2.38
All Patients 327
Low 230
Intermediate 53
High 44
Age
< 50
All Patients 66
Low risk 47
Intermediate risk 13
High risk 6
≥ 50
All Patients 261
Low risk 183
Intermediate risk 40
High risk 38
Menopausal Status
Premenopausal
All Patients 79
Low risk 53
Intermediate risk 16
High risk 10
Postmenopausal
All Patients 248
Low risk 177
Intermediate risk 37
High risk 34
Lesion Size
≤ 10 mm
All Patients 260
Low risk 184
Intermediate risk 37
High risk 39
> 10 mm
All Patients 67
Low risk 46
Intermediate risk 16
High risk 5
Margin
< 5 mm
All Patients 113
Low risk 80
Intermediate risk 18
High risk 15
5 - 9 mm
All Patients 144
Low risk 99
Intermediate risk 22
High risk 23
≥ 10 mm
All Patients 70
Low risk 51
Intermediate risk 13
High risk 6
Grade (Present Study)
Low / Intermediate
All Patients 216
Low risk 179
Intermediate risk 28
High risk 9
High
All Patients 111
Low risk 51
Intermediate risk 25
High risk 35
Cohort (Local Grade)
Cohort 1
All Patients 273
Low risk 210
Intermediate risk 40
High risk 23
Cohort 2
All Patients 54
Low risk 20
Intermediate risk 13
High risk 21
VNPI Group
Low VNPI
All Patients 203
Low risk 171
Intermediate risk 23
High risk 9
Intermediate VNPI
All Patients 124
Low risk 59
Intermediate risk 30
High risk 35
0 10 20 30 40 50 60 70
10 Year Risk of IBE (%)
0.2 0.5 1 2 4 8 16 32
DCIS Score Hazard Ratio

Supplementary Figure 7. Distribution of the DCIS Score according to clinical and pathologic characteristics, including scatter plots and the frequency in each prespecified risk group. Spearman rank correlations and 95% confidence intervals (CI) were calculated for the DCIS Score versus each characteristic, where the characteristics were treated as ordinal categorical variables except in the cases of age, tumor size, and percent comedo necrosis, where the continuous variable was used.

A. Distribution of the DCIS Score according to age group.

B. Distribution of the DCIS Score according to menopausal status.

C. Distribution of the DCIS Score according to tumor size.

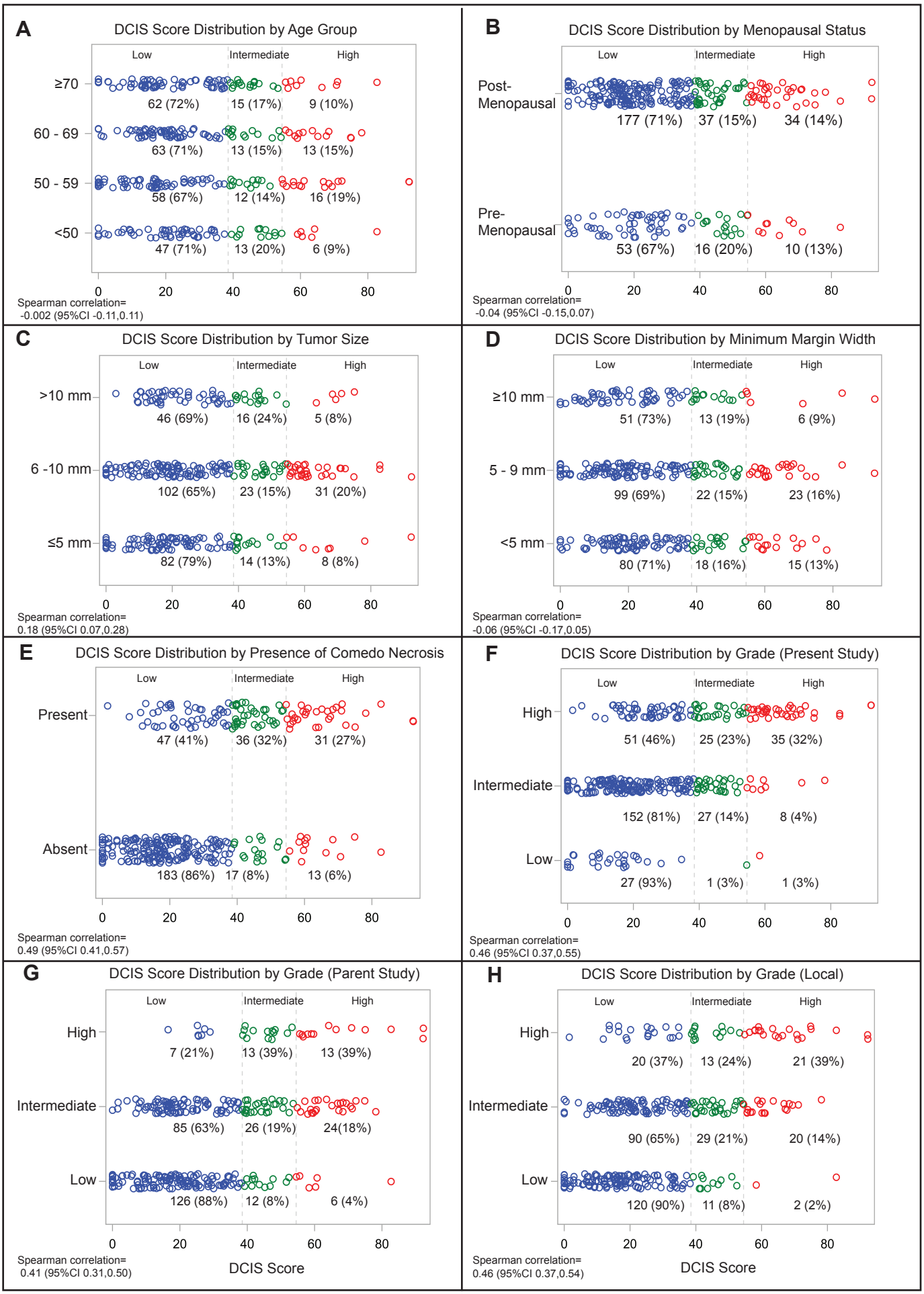
D. Distribution of the DCIS Score according to minimum negative margin width.

E. Distribution of the DCIS Score according to presence of comedo necrosis.

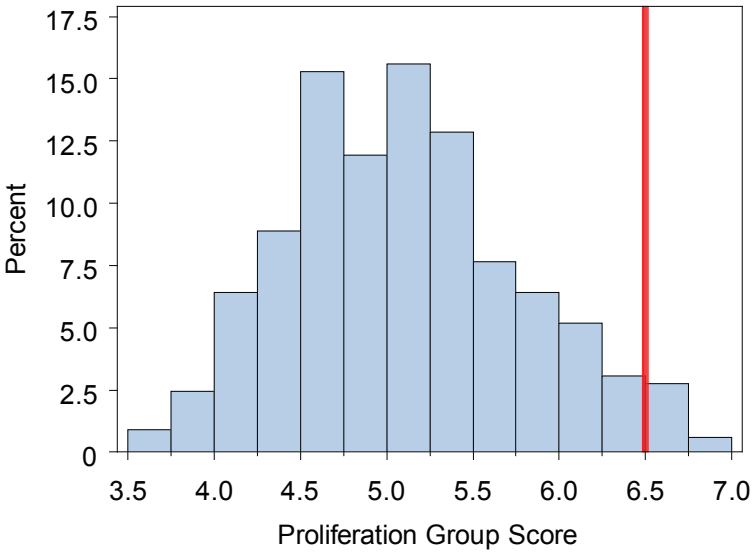
F. Distribution of the DCIS Score according to central grade for the present study.

G. Distribution of the DCIS Score according to central grade for the parent study.

H. Distribution of the DCIS Score according to local grade for the parent study.

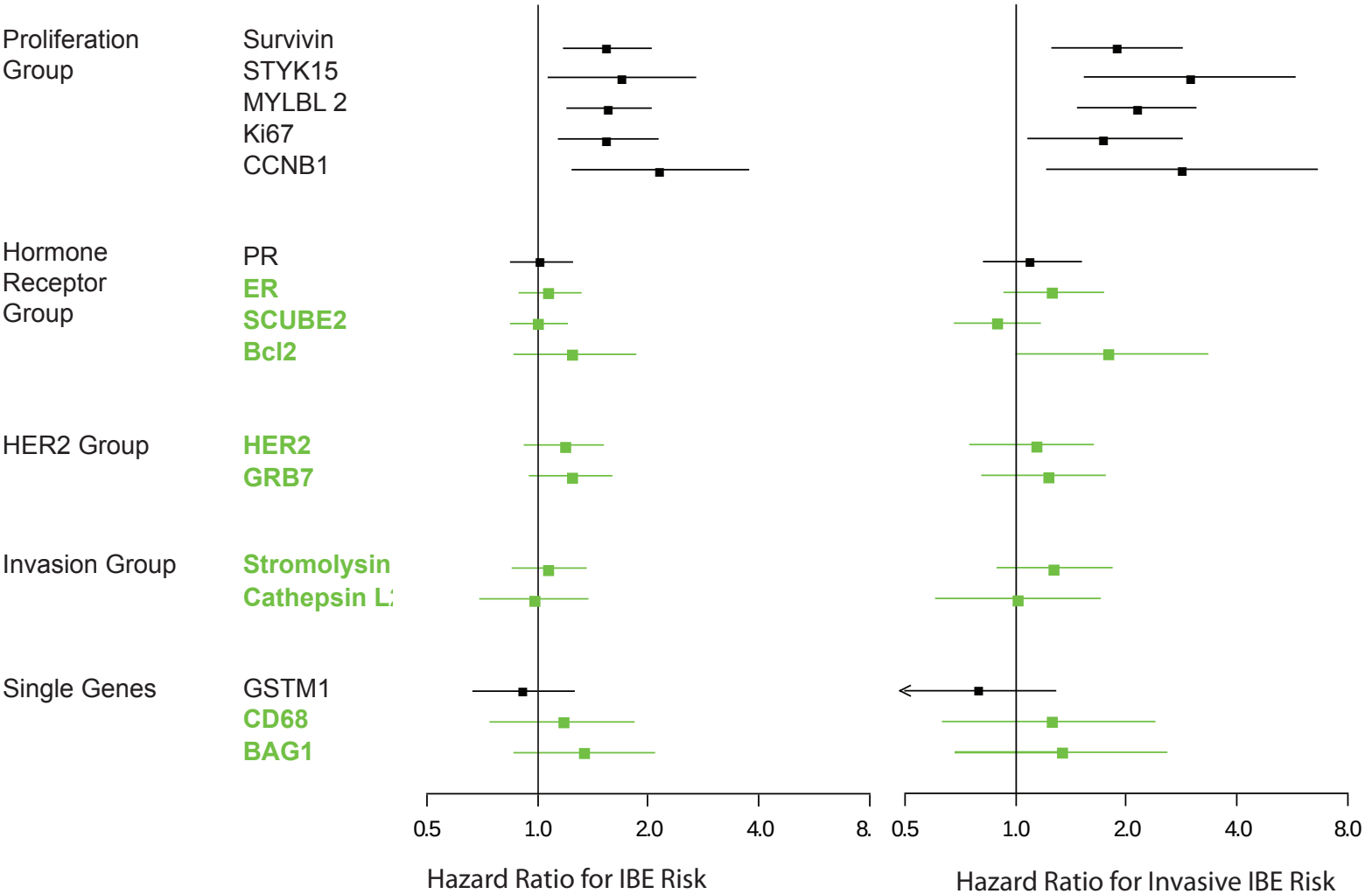

A
DCIS Score Distribution by Age Group
Low
Intermediate
High
≥70
62 (72%)
15 (17%)
9 (10%)
60 - 69
63 (71%)
13 (15%)
13 (15%)
50 - 59
58 (67%)
12 (14%)
16 (19%)
<50
47 (71%)
13 (20%)
6 (9%)
0
20
40
60
80
Spearman correlation=
-0.002 (95%CI -0.11,0.11)
B
DCIS Score Distribution by Menopausal Status
Post-
Menopausal
177 (71%)
37 (15%)
34 (14%)
Pre-
Menopausal
53 (67%)
16 (20%)
10 (13%)
Spearman correlation=
-0.04 (95%CI -0.15,0.07)
C
DCIS Score Distribution by Tumor Size
>10 mm
46 (69%)
16 (24%)
5 (8%)
6 -10 mm
102 (65%)
23 (15%)
31 (20%)
≤5 mm
82 (79%)
14 (13%)
8 (8%)
Spearman correlation=
0.18 (95%CI 0.07,0.28)
D
DCIS Score Distribution by Minimum Margin Width
≥10 mm
51 (73%)
13 (19%)
6 (9%)
5 - 9 mm
99 (69%)
22 (15%)
23 (16%)
<5 mm
80 (71%)
18 (16%)
15 (13%)
Spearman correlation=
-0.06 (95%CI -0.17,0.05)
E
DCIS Score Distribution by Presence of Comedo Necrosis
Present
47 (41%)
36 (32%)
31 (27%)
Absent
183 (86%)
17 (8%)
13 (6%)
Spearman correlation=
0.49 (95%CI 0.41,0.57)
F
DCIS Score Distribution by Grade (Present Study)
High
51 (46%)
25 (23%)
35 (32%)
Intermediate
152 (81%)
27 (14%)
8 (4%)
Low
27 (93%)
1 (3%)
1 (3%)
Spearman correlation=
0.46 (95%CI 0.37,0.55)
G
DCIS Score Distribution by Grade (Parent Study)
High
7 (21%)
13 (39%)
13 (39%)
Intermediate
85 (63%)
26 (19%)
24(18%)
Low
126 (88%)
12 (8%)
6 (4%)
Spearman correlation=
0.41 (95%CI 0.31,0.50)
DCIS Score
H
DCIS Score Distribution by Grade (Local)
High
20 (37%)
13 (24%)
21 (39%)
Intermediate
90 (65%)
29 (21%)
20 (14%)
Low
120 (90%)
11 (8%)
2 (2%)
Spearman correlation=
0.46 (95%CI 0.37,0.54)
DCIS Score

Supplementary Figure 8. Distribution of the proliferation group scores in the present study for the overall group of 327 patients. The reference line of 6.5 indicates the threshold used in the prespecified calculation of the Recurrence Score. In contrast, no threshold was used for the prespecified calculation for the DCIS Score for the present study. Distribution of the proliferation group scores was such that 96.9% (310/320) of the hormone receptor positive patients had a proliferation group score of 6.5 or less.


17.5
15.0
12.5
10.0
7.5
5.0
2.5
0
Percent
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
Proliferation Group Score

Supplementary Figure 9. The forest plot shows the hazard ratios for ipsilateral breast event (IBE) risk (left side of the figure) and invasive IBE risk (right side of the figure) for each of the individual genes in the Onco*type* DX breast cancer assay (excluding the five reference genes). The seven cancer-related genes prespecified as included in the DCIS Score are shown in black. The remaining nine genes (included in the Recurrence Score, but not included in the DCIS Score) are shown in green. The hazard ratios (with 95% confidence intervals) are calculated for a one unit difference (approximately doubling) in cycle threshold (C_T) for gene expression level.

Abbreviations: Survivin = BIRC5; STK15 = aurora kinase A; MYBL2 = v-myb myeloblastosis viral oncogene homolog (avian)-like 2; Ki67 = MKI67; CCNB1 = cyclin B1; PR = progesterone receptor; ER = estrogen receptor; SCUBE2 = signal peptide, CUB domain, EGF-like 2; BCL2 = B-cell CLL/lymphoma 2; HER2 = human epidermal growth factor receptor 2; GRB7 = growth factor receptor-bound protein 7; MMP11 = stromolysin; CTSL2 = cathepsin L2; GSTM1 = glutathione S-transferase M1; CD68 = CD68 molecule; BAG1 = BCL2-associated athanogene.


Proliferation Group
Survivin
STYK15
MYLBL 2
Ki67
CCNB1
Hormone Receptor Group
PR
ER
SCUBE2
Bcl2
HER2 Group
HER2
GRB7
Invasion Group
Stromolysin
Cathepsin L
Single Genes
GSTM1
CD68
BAG1
0.5
1.0
2.0
4.0
8.
Hazard Ratio for IBE Risk
0.5
1.0
2.0
4.0
8.0
Hazard Ratio for Invasive IBE Risk