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Validation of a Ductal Carcinoma *In Situ* Biomarker Profile for Risk of Recurrence after Breast-Conserving Surgery with and without Radiotherapy

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ABSTRACT

Purpose: A major challenge in ductal carcinoma *in situ* (DCIS) treatment is selection of the most appropriate therapeutic approach for individual patients. We conducted an external prospective–retrospective clinical validation of a DCIS biologic risk signature, DCISionRT, in a population-based observational cohort of women diagnosed with DCIS and treated with breast-conserving surgery (BCS).

Experimental Design: Participants were 455 health plan members of Kaiser Permanente Northwest diagnosed with DCIS and treated with BCS with or without radiotherapy from 1990 to 2007. The biologic signature combined seven protein tumor markers assessed in formalin-fixed, paraffin-embedded tumor tissue with four clinicopathologic factors to provide a DCISionRT test result, termed decision score (DS). Cox regression and Kaplan–Meier analysis were used to measure the association of the DS, continuous (linear) or categorical (DS ≤ 3 vs. DS > 3), and subsequent total ipsilateral breast events and invasive ipsilateral breast events at least 6 months after initial surgery.

Results: In Cox regression, the continuous and categorical DS variables were positively associated with total and invasive breast event risk after adjustment for radiotherapy. In a subset analysis by treatment group, categorical Kaplan–Meier analyses showed at least 2-fold differences in 10-year risk of total breast events between the elevated-risk and low-risk DS categories.

Conclusions: In this first external validation study of the DCISionRT test, the DS was prognostic for the risk of later breast events for women diagnosed with DCIS, following BCS.

Introduction

The incidence of ductal carcinoma *in situ* (DCIS), a preinvasive tumor of the breast, has increased sharply since the 1980s due in large part to the advent of widespread and improved mammographic screening programs (1). Nearly 65,000 new DCIS cases were diagnosed in the United States in 2015 (2). Current treatment strategies range widely from mastectomy or breast-conserving surgery (BCS) with radiotherapy to BCS or observation alone (3–5). A meta-analysis of four randomized clinical trials for DCIS revealed that adjuvant radiotherapy further decreased the risk of 10-year local recurrence after BCS by approximately 50% but did not improve breast cancer–specific survival (4). Ten-year breast cancer–specific mortality is approximately 2% with or without adjuvant radiotherapy (4, 6).

Selection of the optimal treatment for individual women is a topic of active research with the goal of understanding the risk of recurrence (7, 8). In the United States, the majority of patients with DCIS are treated with BCS plus radiotherapy (43%), BCS alone (26%), or mastectomy (24%; ref. 5). However, there have been significant differences in treatment patterns over time and across geographic regions (3, 5, 9). While clinicopathologic factors, such as palpable mass, larger size, higher grade, involved margins, and younger diagnosis (age < 50 years), are associated with poor prognosis (10), they lack the power to accurately assess individual risk or identify which patients will have treatment benefit (11–13). The recent 12-year update of the RTOG 9804 study demonstrated a benefit for radiotherapy even in "good" clinicopathologic risk patients (14). An NIH consensus statement summarized this long-standing need, calling for the development of risk assessment tools combining clinicopathologic and biological factors to improve decision making (7).

The DCISionRT DCIS test (PreludeDx) provides a single integrated risk assessment score by combining seven monoclonal protein markers assessed in formalin-fixed, paraffin-embedded (FFPE) tumor tissue with four clinicopathologic factors (15). The biologic signature was designed to provide a 10-year recurrence/progression risk assessment for patients with DCIS following BCS. We performed a clinical validation study of the test within a population-based cohort of women diagnosed with DCIS in the Kaiser Permanente Northwest (KPNW) integrated health plan. We measured the strength of the association between the decision score (DS) and subsequent clinical outcomes, overall and by treatment group.

Materials and Methods

Study design and objectives

This study employed a prospective–retrospective design (16) in which an analytically validated assay system DCISionRT (pronounced

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Translational Relevance

A major challenge in treatment of ductal carcinoma *in situ* (DCIS) is selection of the therapeutic approach for individual patients. Survival is excellent for patients diagnosed with DCIS regardless of treatment; however, risk of subsequent DCIS or invasive breast cancer remains difficult to assess for the individual. Current treatment strategies range widely from mastectomy or breast-conserving surgery (BCS) with radiotherapy to BCS or observation alone. DCISionRT, a biologic signature, was developed to predict radiotherapy benefit and assess recurrence risk in women diagnosed with DCIS who were treated with BCS. In this first external validation study of the DCISionRT test, the test was prognostic for risk of later breast events. This study adds to the literature supporting use of the DCISionRT test in clinical practice to individualize treatment of women diagnosed with DCIS.

Decision RT) was applied to archived tissue specimens with testing personnel blinded to patient outcome. Evaluation of clinical performance followed a protocol with prespecified endpoints and statistical methods. The test combined information from seven protein tumor biomarkers (17–20) and four other factors (age at diagnosis, tumor size, palpability, and surgical margin status) and produced a DS individualized for each patient on a scale of 0–10. Study approval was obtained from the KPNW Institutional Review Board.

An "invasive breast event" (InvBE) was defined as either a subsequent ipsilateral local or regional invasive breast cancer diagnosis or a metastatic event subsequent to the primary ipsilateral DCIS event. A "total breast event" (TotBE) was defined as either a subsequent ipsilateral DCIS event or an InvBE. We used the first qualifying breast event at least 6 months after the primary DCIS diagnosis as the study outcome (6, 21). A "contralateral breast event" (contralateral BE) was defined as a subsequent breast event (either DCIS or invasive cancer) in the other breast. Any metastatic event occurring after an intervening contralateral BE was censored.

The prospective statistical analysis plan (SAP) included prespecified primary and secondary objectives to determine whether the DS continuous score or a DS categorical classifier (DS $\leq$ 3 vs. DS $>$ 3), respectively, were associated with risk of TotBE adjusted for radiotherapy in all evaluable patients. An exploratory objective was to determine whether categorical DS was associated with risk of TotBE in patients treated with BCS without radiotherapy. In *post hoc* analyses, we assessed the association between continuous and categorical DS with InvBE risk adjusted for radiotherapy in all evaluable patients and with continuous and categorical DS with InvBE and TotBE risk adjusted for radiotherapy in the subset of evaluable patients with negative surgical margins after BCS.

The SAP also included prespecified primary, secondary, and exploratory objectives to determine whether an alternative invasive-only risk signature (continuous and categorical scores) was associated with risk of InvBE, mirroring those of the DS analyses (Supplementary Table S4). The invasive-only risk signature was not commercialized, and we include it in this report only because it was part of the prespecified SAP.

Other prespecified analyses assessed both the association of DS with TotBE risk in the subset of evaluable patients who did not receive endocrine therapy and the benefit of DS in the context of clinicopathology. Specifically, the ability of the DS to predict outcomes above and beyond clinicopathology was tested in all evaluable patients using multivariable Cox regression for TotBE risk in relation to DS (categorical and continuous) and a panel of clinicopathologic factors modeled individually, in combination, and, in *post hoc* analysis, as a combined weighted score of clinicopathologic factors and treatment, using the predefined weights employed by the Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY) DCIS risk nomogram (22).

Patient population

Using the KPNW Tumor Registry, we identified women age 26 years or older diagnosed from 1990 to 2007 with histologically confirmed DCIS and treated with BCS with or without adjuvant radiotherapy. All eligible subjects were enrolled in the health plan at DCIS diagnosis and for at least 6 months afterward with no invasive cancer diagnosis or suspicious mammograms during that period. Patients had no known prior *in situ* or invasive breast cancer and no previous treatment for breast disease.

Data collection

Clinical data were obtained from electronic and/or paper medical records. Pathology data were obtained from pathology reports augmented by central pathology review. For quality assurance purposes, all records with a TotBE and 10% of all other records were reabstracted and reviewed for accuracy. DCIS diagnosis and study eligibility were confirmed by central pathology review at KPNW and PreludeDx. A KPNW pathologist selected representative FFPE tumor tissue specimens. Tissue slides, abstracted pathology data, and associated clinical pathology reports were deidentified and blinded to outcome and provided to PreludeDx via a data transfer authority.

Surgical margin status was defined as "positive" if tumor cells were identified at the inked resection margin and "negative" if no tumor cells were identified at the inked resection margin, consistent with the randomized DCIS trials for radiotherapy (4). Multifocal lesions were defined as those having more than one distinct focus of DCIS with at least 5.0 mm of intervening benign tissue. Tumor size was based on the greatest possible extent of the lesion from pathology reports accounting for reexcision.

The DS was calculated using seven IHC-evaluated biomarkers (COX-2, FOXA1, HER2, Ki-67, p16/INK4A, PR, and SIAH2) plus four clinicopathologic factors (age at diagnosis, tumor size, palpability, and surgical margin status) using a predetermined nonlinear algorithm designed to estimate the likelihood of TotBE risk for 10 years following treatment by BCS with or without adjuvant radiotherapy. The DS is a continuous number ranging from 0 to 10 with higher scores reflecting higher risk (15). Staining of protein biomarkers in the FFPE tissues was performed at the Clinical Laboratory Improvement Amendments–certified PreludeDx laboratory within 2 weeks of receipt, with scoring methods as described previously (15) and in the Supplementary Materials and Methods. The testing was performed on intact FFPE tissue mounted slides, which preserved tissue architecture. This enabled the test to evaluate the protein expression only in epithelial tissue in the ducts containing DCIS while excluding contaminating effects from other tissue cellularity, such as stromal tissue, which may have strikingly different expression profiles (Supplementary Materials and Methods). PreludeDx transmitted a biomarker assay result dataset to KPNW that was combined with clinical and outcome data for final data analysis. The development and cross-validation of the proprietary nonlinear risk algorithm utilized to calculate DS has been described previously (15).

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Data analysis

A KPNW Center for Health Research biostatistician (M.C. Leo) executed a predefined SAP agreed to by PreludeDx and KPNW. Data analysis was performed using the R statistical package (23) and replicated in STATA (24). Patient characteristics were summarized for evaluable and excluded eligible cohorts by count and frequency. Clinicopathologic differences by radiation treatment group for the evaluable cohort were determined by *t* test or by Fisher exact test. We used Cox proportional hazards regression to compute HRs and 95% confidence intervals (CI) for continuous and categorical DS in relation to TotBE and InvBE risks, after adjusting for the effect of radiotherapy. For categorical DS, we used a predefined threshold of DS > 3 to define the elevated risk group. Categorical and continuous DS in relation to TotBE risk assessment were also determined using Cox proportional hazards regression while adjusting for treatment and clinicopathology factors. The treatment and clinicopathology factors consisted of radiation and endocrine treatment, age, number of excisions, surgical margins, tumor grade, presentation (clinical vs. routine mammogram), and tumor necrosis. Continuous and categorical DS were first analyzed adjusted for the effects of treatment and clinicopathology as separate covariates. In addition, these treatment and clinicopathologic factors were adjusted for in the analysis by weighting and summing them into a continuous term on the basis of a predefined weighting from the MSKCC nomogram (22), here called "modified MSKCC score." The modified MSKCC score was based on the DCIS nomogram and used a definition of treatment and clinicopathologic factors and corresponding weights consistent with the nomogram, except in the case of margin status (22). The method for defining surgical margin status was adapted for this study, as the MKSCC nomogram defines a clear surgical margin as 2 mm or greater, while in our study negative surgical margin status was defined as no ink on tumor and positive margin status was defined as ink on tumor. Pearson correlation was used to assess the relationship between DS and the modified MSKCC score. Continuous and categorical DS stratified by radiotherapy groups were assessed by Cox proportional hazards analysis. Kaplan–Meier analysis was used to compute 10-year breast event risks for categorical DS risk groups and ranges of DS. Average 10-year breast event risks were also calculated by Kaplan–Meier analysis for selected intervals of DS. These average 10-year risks were plotted as a function of DS and overlaid on the previously published continuous DS risk curve plot for comparison. The percentage distribution of categorical DS within individual, combinations, and panels of clinicopathologic factors was also described. In the case of RTOG 9804 criteria, a negative margin status of no ink on tumor was used and termed RTOG 9804 "like" criteria. All inferential tests were evaluated using a two-tailed alpha level of 0.05. Study results are reported consistent with REMARK guidelines (25). See Supplementary Materials and Methods for more details on patient selection, data collection, laboratory methods, and data analysis.

Results

Patient population

We identified 685 women diagnosed from 1990 to 2007 with histologically confirmed DCIS and treated with BCS without subsequent mastectomy. FFPE blocks were available for 515 (85%) of the 608 women meeting study eligibility criteria (Supplementary Fig. S1). Of these 515 eligible patients with FFPE blocks available, 14 patients were missing clinicopathologic factors used by DS and 21 eligible patients were excluded for other reasons (Supplementary Fig. S1). Of the remaining 480 patients, 455 (95%) had sufficient tissue available for DCISionRT assay testing (e.g., eight 3 μm sections with two ducts or 1 mm of DCIS). There were no other assay failures in specimens with sufficient tissue.

Characteristics of the 455 patients in the evaluable study population and the 153 excluded eligible patients were generally similar (Supplementary Table S1). Among the evaluable cases with confirmed DCIS and complete DCISionRT results, 78 women (17%) were treated with BCS without radiotherapy and 377 (83%) were treated with BCS plus radiotherapy (**Table 1**). Overall, 24% were prescribed adjuvant endocrine therapy (average duration 4.2 years). Median follow-up time was 10.4 years. There were minor clinicopathologic variations between patients treated with BCS without radiotherapy and BCS plus radiotherapy. Patients receiving BCS without radiotherapy were more likely to be 60 years of age and older, more likely to have lower tumor grade, and less likely to have tumor necrosis. They were also less likely to receive endocrine therapy and more likely to have positive surgical margins (due primarily to documented patient refusal of additional surgical resections). Unless otherwise specified, results are reported below for the evaluable population.

DS and DS risk groups in relation to breast event risk

Our study's primary and secondary aims were to assess the association between continuous DS (linear relationship) and categorical DS with TotBE risk adjusted for the effect of radiotherapy in all patients, respectively (**Table 2**). After adjusting for radiotherapy, the association of DS with increasing TotBE risk was statistically significant in a multivariable Cox proportional hazards analysis with up to 25 years follow-up (**Table 2**). Increasing continuous DS was positively associated with increasing TotBE risk after adjustment for radiotherapy (HR, 1.76 per 5 DS units; 95% CI, 1.16–2.70). In this study, categorical DS divided the population into a DS low risk group (41.8%) and a DS elevated risk group (58.2%). Patients in the categorical DS elevated risk group were at increased TotBE risk compared with the DS low risk group after adjusting for radiotherapy (HR, 2.03; 95% CI, 1.12–3.70). Similarly, after adjusting for radiotherapy, both continuous DS and categorical DS were positively associated with increased InvBE risk (continuous HR = 1.76 per 5 DS units, 95% CI, 1.05–3.18; and categorical HR = 2.14; 95% CI, 1.00–4.59, respectively; **Table 2**).

In further analyses, categorical and continuous DS were assessed after adjusting for both adjuvant treatments and clinicopathology factors. Patients in the categorical DS elevated risk group were at significantly increased TotBE risk after adjusting for treatment and clinicopathology as eight individual factors (HR, 1.86; 95% CI, 1.00–3.46; **Table 3**). In a similar analysis for continuous DS, while adjusting for treatment and clinicopathology as eight individual factors, the association was nonsignificant, but the HR point estimate was positive (HR, 1.47 per 5 DS units; 95% CI, 0.90–2.39). In a separate analysis, the treatment and clinicopathology were weighted and combined into a continuous factor (modified MSKCC score). The continuous DS was predictive for TotBE risk after adjusting for the modified MSKCC score (HR, 1.58 per 5 units; 95% CI, 1.04–2.38; $P = 0.03$).

We assessed the percentage of patients in the DS low and elevated risk groups in a series of individual and combined categorical clinicopathologic factors used for DCIS risk assessment (**Table 4**). Approximately 50% of patients with individual clinicopathologic factors considered low risk (negative margins, lower grade, smaller size, no necrosis, and negative family history) were classified into the DS elevated risk group. Of patients meeting RTOG 9804 "like" risk criteria (negative margins, screen detected, nonpalpable, less than 2.5 cm, and grade 1–2), 49% were classified into the DS elevated risk group.

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Table 1. Clinicopathologic and breast event characteristics of evaluable patients, overall and by treatment cohort.

Characteristic	No radiotherapy *N* = 78 *N* (%)	Radiotherapy *N* = 377 *N* (%)	All evaluable cases *N* = 455 *N* (%)
Adjuvant endocrine therapy			
No	63 (80.8)	281 (74.5)	344 (75.6)
Yes	14 (18.0)	94 (24.9)	108 (23.7)
Unknown	1 (1.3)	2 (0.5)	3 (0.7)
Year of DCIS diagnosis			
1995 and prior	24 (30.8)	78 (20.7)	102 (22.4)
1996 and after	54 (69.2)	299 (79.3)	353 (77.6)
Age at DCIS diagnosis[a]			
<50	9 (11.5)	82 (21.8)	91 (20.0)
50–59	18 (23.1)	119 (31.6)	137 (30.1)
60–69	21 (26.9)	99 (26.3)	120 (26.4)
70+	30 (38.5)	77 (20.4)	107 (23.5)
Family history of breast cancer			
No	44 (56.4)	196 (52.0)	240 (52.8)
Yes	32 (41.0)	179 (47.5)	211 (46.4)
Unknown	2 (2.6)	2 (0.5)	4 (0.9)
Presentation			
Routine mammogram	69 (88.5)	340 (90.2)	409 (89.9)
Clinical	9 (11.5)	37 (9.8)	46 (10.1)
Palpability			
Negative	70 (89.7)	345 (90.2)	415 (91.2)
Positive	8 (10.3)	37 (9.8)	40 (8.8)
Tumor grade[a]			
Low	23 (29.5)	41 (10.9)	64 (14.1)
Intermediate	37 (47.4)	163 (43.2)	200 (44.0)
High	18 (23.1)	172 (45.6)	190 (41.8)
Unknown	0 (0.0)	1 (0.3)	1 (0.2)
Tumor size			
5 mm or less	27 (34.6)	109 (28.9)	136 (29.9)
6–10 mm	16 (20.5)	77 (20.4)	93 (20.4)
≥10 mm	10 (12.8)	72 (19.1)	82 (18.0)
Unknown	25 (32.1)	119 (31.6)	144 (31.7)
Surgical margin status[a]			
Negative	69 (88.5)	364 (96.6)	433 (95.2)
Positive	9 (11.5)	13 (3.5)	22 (4.8)
Tumor necrosis[a]			
Absent	34 (43.6)	91 (24.1)	125 (27.5)
Present	44 (56.4)	286 (75.9)	330 (72.5)
Surgical excisions			
1	58 (74.4)	265 (70.3)	323 (71.0)
2	17 (21.8)	99 (26.3)	116 (25.5)
3 or more	0 (0.0)	8 (2.1)	8 (1.8)
Unknown	3 (1.3)	5 (1.3)	8 (1.8)
Ipsilateral outcome (first documented)			
No ipsilateral event	64 (82.0)	338 (89.7)	402 (88.4)
Ipsilateral DCIS	6 (7.7)	14 (3.7)	20 (4.4)
Ipsilateral invasive BC	6 (7.7)	20 (5.3)	26 (5.7)
Metastasis	2 (2.6)	5 (1.3)	7 (1.5)
Contralateral outcome (first documented)			
No contralateral event	77 (98.7)	359 (95.2)	436 (95.8)
Contralateral DCIS	0 (0.0)	7 (1.9)	7 (1.5)
Invasive contralateral BC	1 (1.3)	11 (2.9)	12 (2.6)

Note: Summary statistics of clinicopathologic factors and ipsilateral and contralateral BEs for the evaluable patient population, overall and by radiotherapy treatment status.

Abbreviation: BC, breast cancer.

[a]Statistically significant difference ($P < 0.05$) between "no radiotherapy" group and "radiotherapy" group.

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Table 2. DCISionRT continuous DS and categorical DS in relation to total and invasive breast event risk adjusted for radiotherapy.

Treatment group/ risk signature	TotBE No. subjects/ recurrences	TotBE HR (95% CI)*	InvBE No. subjects/ recurrences	InvBE HR (95% CI)*
All evaluable patients with and without radiotherapy				
DS per 5 units	455/53	1.76 (1.16–2.70)*	455/33	1.76 (1.05–3.18)*
Low risk group ≤ 3	190/16	Reference	190/10	Reference
Elevated risk group > 3	265/37	2.03 (1.12–3.70)*	265/23	2.14 (1.00–4.59)*

Note: Multivariable Cox proportional hazards analyses were completed for the full study period (up to 25 years of follow-up). DS was analyzed with radiotherapy as a covariate (*) shown in all evaluable subjects ± radiotherapy for continuous DS and DS risk groups. The number of events within 10 years was 38 for TotBE and 21 for InvBE.
Abbreviations: DS, Decision score; InvBE, invasive breast events; TotBE, total breast events.

In exploratory analyses, although the study was not powered to detect significant differences within radiotherapy groups, we investigated the association of DS with subsequent ipsilateral breast event risk by radiotherapy groups. In women treated without radiotherapy, increasing DS was significantly associated with increasing TotBE (HR, 2.70 per 5 DS units; 95% CI, 1.16–6.19) and InvBE risk (HR, 4.0 per 5 DS units; 95% CI, 1.33–12.60; **Table 5**). In the subset of patients who did not receive radiotherapy, there were approximately the same percentages of patients in the DS low and elevated risk groups. The association of categorical DS with TotBE and InvBE risk was not significant. However, in this sample, the DS elevated risk group had over twice the number of events as the DS low risk group. In contrast to women treated with BCS without radiotherapy, in the women who received BCS plus radiotherapy the total and invasive event rates were substantially attenuated. In these women treated with radiotherapy, the association of the DS (continuous and categorical) with TotBE and InvBE risk was nonsignificant.

The 10-year absolute risks for select DS intervals were assessed by Kaplan–Meier analysis. These average 10-year risks were plotted versus DS and overlaid onto the published 10-year risk curves as a function of DS (**Fig. 1**). The data points in blue (**Fig. 1**) show the 10-year risks for patients treated with BCS without radiotherapy, while the data points in orange show the risks for patients receiving BCS plus radiotherapy. Among women who received BCS without radiotherapy, the 10-year TotBE risk for the DS elevated risk group was 30% (95% CI, 17%–51%), compared with 10% (95% CI, 3%–29%) in the DS low risk group (**Table 6**), similar to the TotBE risk of 8% (95% CI, 5%–11%) in the overall group of all DCIS treated with radiotherapy. Of those receiving BCS plus radiotherapy, women in the DS elevated risk group had a TotBE risk of 10% (95% CI, 6%–15%) compared with 5% (95% CI, 2%–10%) in the DS low risk group. For BCS without radiotherapy patients, InvBE risk in the DS elevated risk group was 21% (95% CI, 9%–44%); in the DS low risk group it was 5% (95% CI, 1%–30%), similar to the overall study 10-year InvBE risk of 5% (95% CI, 3%–9%)

Table 3. Multivariable analysis of the association of DCISionRT categorical DS and clinicopathologic factors and treatment with total breast event risk.

	TotBE HR (95% CI)	TotBE *P*
Categorical DS, elevated (> 3) vs. low (≤ 3)	1.86 (1.00–3.46)	0.048
Age, < 50 vs. ≥ 50 years	1.24 (0.66–2.32)	0.50
Presentation, clinical vs. screening	1.16 (0.48–2.79)	0.74
Excision no., 3+ vs. 1 or 2	1.11 (0.15–8.37)	0.92
Grade, 3 vs. 1 or 2	0.89 (0.49–1.59)	0.69
Necrosis, present vs. absent	2.58 (1.14–5.84)	0.023
Surgical margin, positive vs. negative	1.74 (0.64–4.75)	0.28
Endocrine therapy, yes vs. no	0.39 (0.15–0.98)	0.045
Radiotherapy, yes vs. no	0.40 (0.21–0.77)	0.006

Note: The association of categorical DS in combination with clinical, pathologic, and treatment factors with total (DCIS and invasive) breast event risk was assessed in the evaluable patient population by Cox proportional hazards analysis ($n = 455$). Negative surgical margin status was defined as no tumor cells identified at the inked resection margin. Endocrine therapy was based on prescribed course of therapy.
Abbreviations: TotBE, total breast events.

Table 4. Categorical DS risk group classification within select clinicopathologic risk assessment categories in evaluable patients with negative surgical margins.

Clinicopathologic factor(s)	Low risk group (DS ≤ 3) %	Low risk group (DS ≤ 3) *N*	Elevated risk group (DS > 3) %	Elevated risk group (DS > 3) *N*
Grade				
Grade 1, 2 (low, intermediate; "lower risk")	50%	124	50%	126
Grade 3 (high)	36%	66	64%	116
Grade and size				
Grade 1 or 2 and size < 1 cm ("lower risk")	59%	67	41%	46
Grade 3 or size ≥ 1 cm	34%	83	66%	161
Family history				
No ("lower risk")	49%	111	51%	117
Yes	37%	75	63%	126
Necrosis				
Absent ("lower risk")	49%	58	51%	61
Present	42%	132	58%	182
RTOG 9804 – like criteria				
"Good" risk ("lower risk")	51%	71	49%	69
Not "good" risk	36%	81	64%	143

Note: Patients were classified by DS risk groups within individual or combinations of clinicopathologic factors that have been associated with subsequent breast event risk. RTOG 9804-like criteria were nonpalpable, screening detected, extent < 2.5 cm, grade 1 or 2, and negative surgical margins (where negative margin status was defined as no tumor cells identified at the inked resection margin). Patient percentages and counts are indicated.
Abbreviations: DS, Decision score; Grade, nuclear grade; RTOG, Radiation Therapy Oncology Group.

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Table 5. DCISionRT continuous DS and categorical DS in relation to total and invasive breast event risk, by radiotherapy.

	Stratified by radiation treatment			
	TotBE		InvBE	
Treatment group	No. subjects/ recurrences	HR (95% CI)*	No. subjects/ recurrences	HR (95% CI)*
Radiotherapy				
DS per 5 units	377/39	1.47 (0.90–2.49)	377/25	1.33 (0.73–2.59)
Low risk group ≤ 3	149/12	Reference	149/8	Reference
Elevated risk group > 3	228/27	1.72 (0.86–3.41)	228/17	1.73 (0.74–4.05)
No radiotherapy				
DS per 5 units	78/14	2.70 (1.16–6.1)	78/8	4.0 (1.33–12.60)
Low risk group ≤ 3	41/4	Reference	41/2	Reference
Elevated risk group >3	37/10	3.04 (0.95–9.73)	37/6	3.80 (0.76–0.18.92)

Note: Multivariable Cox proportional hazards analyses were completed for the full study period (up to 25 years of follow-up). The number of events within 10 years was 38 for TotBE and 21 for InvBE.
Abbreviations: DS, Decision score; InvBE, Invasive breast events; TotBE, total breast events.

for patients treated with radiotherapy (**Table 6**). In comparison, among women treated with BCS plus radiotherapy, patients in the DS low risk group had a 10-year InvBE risk of 3% (95% CI, 1%–9%), while the risk for patients in the elevated risk group was 6% (95% CI, 3%–10%; **Table 6**).

All patients in the DS low risk group had negative surgical margins. Overall, only 22 (4.8%) of 455 patients had positive margins. Excluding positive margin patients from analysis resulted in no change in calculated TotBE or InvBE risk with one nonsignificant exception. The DS elevated risk group treated with BCS without radiotherapy had a 10-year InvBE risk of 21% (95% CI, 9%–44%) including the women with positive margins; excluding positive margin patients yielded a 10-year InvBE risk of 12% (95% CI, 3%–41%) in the same group (**Fig. 1**; Supplementary Table S2).

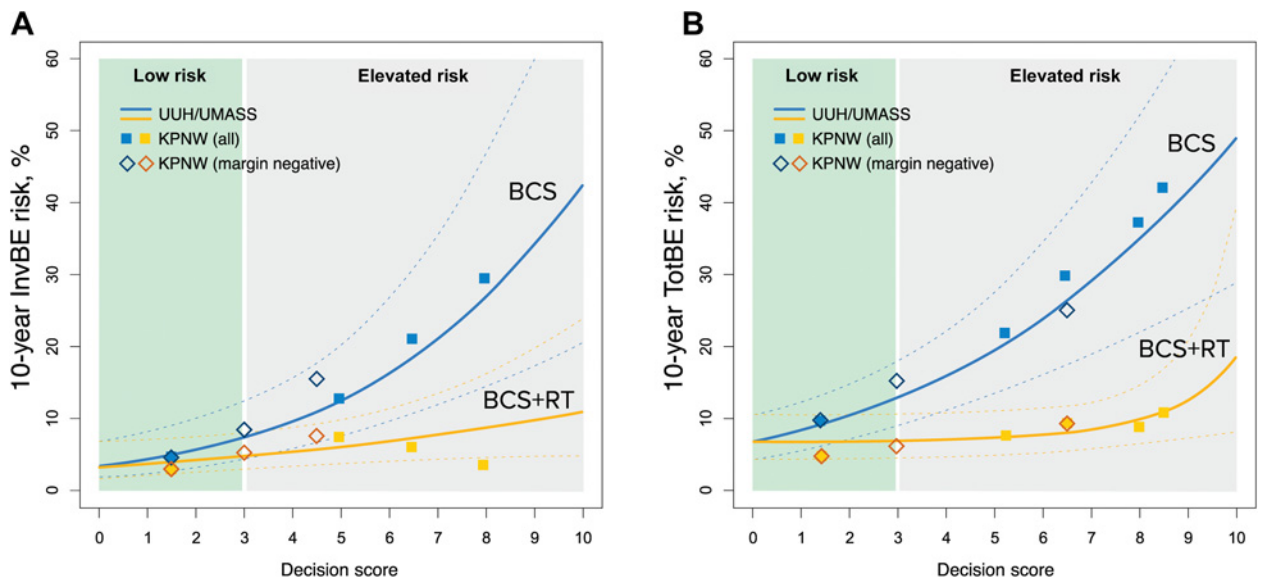

Figure 1.
Ten-year breast event risk in KPNW patients as a function of DS compared with published continuous risk curves for DS from Uppsala University Hospital/University of Massachusetts (UUH/UMASS) cohort, by receipt of radiotherapy (RT). **A,** The average 10-year risk of InvBE for DS intervals from the KPNW patient cohort are compared with the 10-year continuous risk curves as a function of DS that were previously published for the UUH/UMASS cohorts (15). The solid blue line represents risk for patients who received BCS alone and the solid orange line represents risk for patients who received BCS and radiotherapy. The dotted lines represent 95% CIs of the corresponding solid lines. The shaded green area denotes the low risk group (DS ≤ 3) while the shaded gray area represents the elevated risk group (DS > 3). Average 10-year InvBE risk was calculated for intervals of DS by Kaplan–Meier analysis (points). Patients in the full evaluable KPNW patient cohort are indicated by closed squares. Patients in the evaluable KPNW patient cohort excluding patients with positive margins are indicated by open diamonds. **B,** The average 10-year risk of TotBE for DS intervals from the KPNW patient cohort are compared with the 10-year continuous risk curves as a function of DS that were previously published for the UUH/UMASS cohorts (15). The solid blue line represents patients who received BCS alone and the solid orange line indicates risk for patients who received BCS and radiotherapy. The dotted lines represent 95% CIs of the corresponding solid line. The shaded green area denotes the low risk group (DS ≤ 3) while the shaded gray area represents the elevated risk group (DS > 3). Average 10-year TotBE risk was calculated for intervals of DS by Kaplan–Meier analysis (points). Patients in the full evaluable KPNW patient cohort are indicated by closed squares. Patients in the evaluable KPNW patient cohort excluding patients with positive margins are indicated by open diamonds.

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Table 6. Ten-year risks for overall study and DS risk groups in all evaluable patients.

	TotBE			
	No radiotherapy		Radiotherapy	
Risk group	Risk (95% CI)	*N*	Risk (95% CI)	*N*
Overall study population	20% (12–32%)	78	8% (5–11%)	377
Low risk group (DS ≤ 3)	10% (3–29%)	41	5% (2–10%)	149
Elevated risk group (DS > 3)	30% (17–51%)	37	10% (6–15%)	228

	InvBE			
	No radiotherapy		Radiotherapy	
Risk group	Risk (95% CI)	*N*	Risk (95% CI)	*N*
Overall study population	13% (6–27%)	78	5% (3–8%)	377
Low risk group (DS ≤ 3)	5% (1–30%)	41	3% (1–9%)	149
Elevated risk group (DS > 3)	21% (9–44%)	37	6% (3–10%)	228

Note: The 10-year breast event risks were determined by Kaplan–Meier analysis for TotBE and InvBE risks with 95% CIs and patient counts. The 10-year risks for the overall study population (independent of DCISionRT results) treated with BCS without radiotherapy and BCS plus radiotherapy are presented. Similarly, the 10-year risks for patients in the DCISionRT DS low risk (DS ≤ 3) group and elevated risk (DS > 3) group are presented.

As anticipated, endocrine therapy significantly reduced 10-year risk as assessed in the multivariable analysis with DS and radiotherapy with clinicopathology factors (HR, 0.39; 95% CI, 0.15–0.98). A limited number of patients (overall 24%) received endocrine therapy (**Table 1**). TotBE risk by DS risk group was assessed in the subset of BCS plus radiotherapy patients who did not receive endocrine therapy (Supplementary Table S3). Compared with TotBE risk for the overall BCS plus radiotherapy population, these women had an absolute 1%–2% higher risk in the DS low risk and elevated risk groups, respectively.

Discussion

The goal of DCIS management is to reduce or eliminate TotBE risk and, most importantly, InvBE risk. Current clinical practice guidelines indicate that some patients may be sufficiently low risk so as to derive no clinically meaningful benefit from radiotherapy. The 2010 DCIS meta-analysis reported that about 70% of patients who undergo BCS without radiotherapy do not have any ipsilateral breast events within 10 years (4, 26). However, there is no uniformly adopted approach for risk assessment, and the utility of clinical pathologic assessment to identify low-risk patients remains limited, resulting in both undertreatment and overtreatment for many women (10, 26, 27). This underscores the need for a biomarker assay to improve identification of those patients with DCIS who are at elevated risk and will benefit from radiotherapy, as well as those patients who are at low risk and do not need radiotherapy to avoid a future local recurrence.

This prospective–retrospective independent clinical validation of DCISionRT was conducted using an observational cohort of patients diagnosed with DCIS. In this population, the test was prognostic for subsequent ipsilateral breast event risk after adjusting for radiotherapy. Specifically, in Cox regression analysis assessing the primary and secondary prespecified study endpoints, continuous and categorical DS produced by the DCISionRT test were positively associated with TotBE risk in the KPNW population after adjustment for radiotherapy. Similarly, in *post hoc* analysis, DS was associated with InvBE risk adjusted for radiotherapy.

The magnitude of the average 10-year absolute risks in DS low and elevated risk groups were consistent with other Kaplan–Meier analysis results previously published on observational cohorts from University of Massachusetts/Uppsala University Hospital (ref. 15; **Table 6**). The observed pattern of 10-year ipsilateral breast event risks for different DS cut-off points (e.g., DS from 2–5) were also consistent with the continuous DS risk curves in the earlier publication (ref. 15; **Fig. 1**).

In this population, within the DS elevated risk group, the average 10-year radiotherapy absolute risk differences were 20% (30%–10%) for TotBE risk and 15% (21%–6%) for InvBE risk, which correspond to relative risk differences of about 66% and 71% for TotBE and InvBE. Defining a low-risk group with a small benefit of radiotherapy could possibly help reduce overtreatment in patients with DCIS. In this population, within the DS low risk group, the average 10-year radiotherapy absolute risk differences were 5% (10%–5%) for TotBE risk and 2% (5%–3%) for InvBE risk.

The algorithm was developed with both biomarkers and clinicopathologic factors together, allowing for interactions to maximize the prediction of risk. Consequently, the test result is calculated by a nonlinear risk algorithm, not a weighted linear equation, and therefore it is not possible to directly separate the contributions of the DS clinicopathologic factors from the biomarkers used in the test. However, to indirectly determine the value of the biomarkers relative to clinicopathology, we assessed whether categorical DS was independently associated with TotBE risk after adjusting for clinicopathologic factors (traditional and those used in the test) in addition to radiotherapy. In multivariable modeling, categorical DS was associated with ipsilateral breast event risk after adjustment for individual clinicopathologic factors (**Table 3**; Supplementary Fig. S2). To further evaluate the contribution of the test compared with clinicopathology, the classification of patients into DS low and elevated risk was assessed among patients who had traditionally low-risk clinicopathologic features (grade 1 or 2 DCIS and tumors less than 1 cm in size). The incorporation of the protein biomarkers and the four DS clinicopathologic factors in the DCISionRT algorithm allowed for further risk stratification of patients with traditionally low-risk clinicopathology factors, as 50% of these patients had elevated categorical DS (**Table 4**). This observation is consistent with prior studies showing that some BCS-treated patients with smaller size or lower grade DCIS tumors still had substantial 10-year recurrence in the DS elevated risk group (4, 28). Thus, use of categorical DS may lead to more refined risk estimates than clinicopathology alone.

We also assessed the utility of continuous DS after adjusting for the same eight clinicopathology factors and treatment, where the factors were either a set of individual variables as above or a single combined score. In the analysis that adjusted for clinicopathology and treatment as a set of individual variables, the continuous DS point estimate was similar to the categorical result but not statistically significant. However, the continuous DS did provide additional utility for assessing TotBE risk after adjusting for the modified MSKCC score. The MSKCC DCIS nomogram has been validated in multiple studies (27, 29, 30) and in comparison with another DCIS genomic assay (31). The modified MSKCC score combined the eight clinicopathology and treatment variables into a single variable, reducing the number of covariates from eight to one in the multivariable analysis. Thus, the observed differences in continuous DS after adjusting for clinicopathology and treatment as individual variables or as a single variable may be due to difference in statistical power.

Similar to the MSKCC nomogram, the DS score includes surgical margin status to take into account the impact of a positive surgical margin on breast event risk. While reexcision for patients treated

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without radiotherapy is usual care for those with a positive margin, some patients may refuse further surgery. In this study, all patients in the DS low risk group had negative margins. The absolute 10-year breast event risks by treatment and surgical margin status were consistent with the continuous risk curves that were reported previously (**Fig. 1**). Overall only 22 (4.8%) of 455 patients had positive margins. Excluding positive margin patients from analysis resulted in no change in calculated TotBE or InvBE risk, with one nonsignificant exception. The DS elevated risk group treated without radiotherapy had a 10-year InvBE risk of 21% (95% CI, 9%–44%); excluding positive margin patients yielded a 10-year InvBE risk of 12% (95% CI, 3%–41%) in the same group (**Fig. 1**; Supplementary Table S2). In this study, data were not available to identify the extent of margin clearance in patients with negative margins <2 mm.

Some studies among women not treated with radiotherapy have reported that women with negative clear margins <2 mm are at greater local recurrence risk than women with margins ≥2 mm (32–34). The recent multidisciplinary (SSO-ASTRO-ASCO) consensus guideline on margins for BCS + whole breast radiotherapy in DCIS states that a 2-mm clear negative margin is preferred to minimize the risk of ipsilateral tumor recurrence (32), but negative margins less than 2 mm alone are not an indication for mastectomy or reexcision unless other factors are judged to contribute to an otherwise high local recurrence risk (32). A recent study in a large cohort of patients with DCIS demonstrated that there was no significant difference in the local recurrence rate for patients with negative clear margins <2 mm versus ≥2 mm as long as they were treated with radiotherapy (33, 34). In our study, the average absolute TotBE and InvBE risk for patients in the DS low risk group were clinically low, consistent with the overall study population risk of patients treated with BCS plus radiotherapy. However, for patients in the DS low risk group forgoing radiotherapy, data were unavailable to assess the impact of clear margins <2 mm.

The study's prespecified SAP did not contain stratified analysis by treatment type among the primary and secondary objectives, as the study was not powered to produce statistically significant results in the smaller subgroups. However, we included continuous and categorical DS analyses for those women treated without radiotherapy in relation to TotBE as exploratory objectives. In addition, we conducted parallel analyses for women treated with BCS plus radiotherapy, although we anticipated a dampening of the DS effect given the known benefit of radiotherapy. The subgroup of women treated with BCS without radiotherapy was associated with high continuous and categorical HRs, as illustrated in **Fig. 1** (blue). In patients treated by BCS without radiotherapy, the HRs for continuous DS per 5 DS units were statistically significant and were similar to previously published results for both InvBE (HR = 4.0 vs. 4.2) and TotBE (HR = 2.7 vs. 3.1; ref. 15). In contrast, in the subgroup of women treated with radiotherapy, the categorical and continuous DS were not associated with a significant increase in risk, having low HRs. While not formally tested (due to low power), it is interesting that the separation increases between the continuous risk curves with increasing DS for patients treated without radiotherapy (blue) and with radiotherapy (orange; see **Fig. 1**).

These validation results were obtained from an independent observational study in a community-based population with a membership reflecting the demographic characteristics of the surrounding community. FFPE samples were available for a large proportion of the eligible patients with comprehensive clinical data and long-term outcomes over a period of up to 25 years. The overall study TotBE and InvBE risks are consistent with improved diagnosis and treatment of DCIS at the end of the 20th century (22, 35). Our study's treatment-specific 10-year recurrence/progression outcomes are lower than those shown in the EBCTCG meta-analysis of older randomized clinical trials (4) as this population was predominantly diagnosed and treated after 1995 (78%) according to protocols consistent with those currently in use.

This study had several limitations. The lower event rate for patients treated with radiotherapy and a lower number of patients treated without radiotherapy limited the statistical power for multivariable and subgroup analyses. In accordance with the KPNW DCIS treatment protocol, most patients with DCIS received adjuvant radiotherapy, so there were relatively fewer BCS without radiotherapy patients for analysis. As approximately half the total events were invasive, statistical power was more limited for assessing the association of DS with InvBE. In addition, some patients received endocrine therapy in this study, although the effect on overall study outcomes was minimal. While this study suggests a preferential radiotherapy benefit for patients in the DS elevated risk category, the study was not designed to assess radiotherapy benefit, and some of the observed risk difference between the radiation-treated versus nontreated cohorts may be due to patient selection for treatment, as the study was neither randomized nor strictly rule based. Patients receiving BCS without radiotherapy varied in age, tumor grade, and tumor necrosis status compared with those treated with radiotherapy.

Further research is needed to increase the level of evidence for the DCISionRT test and the effects of some clinicopathologic factors. Recent publications have addressed negative margin clearance, but data on the closeness of clear margins is not available in the pathology reporting for this study. Necrosis, commonly found in DCIS tumors in this study, was a significant risk factor for TotBE but not InvBE in multivariable models. Although necrosis has been implicated as a risk factor in some studies, a standard operational definition is lacking. This is exemplified by the widely variable definitions of comedo necrosis provided by 35 breast pathologists in 20 different institutions (36). A separate prospective–retrospective validation in a randomized clinical trial population treated with BCS without radiotherapy and BCS plus radiotherapy is in process to increase the level of evidence for this test and to determine the degree of relative risk reduction as a function of DS.

In conclusion, a major challenge in DCIS treatment today is selection of the most appropriate therapeutic approach for individual patients. Here, in a cohort of women representative of modern treatment practice, a prospective–retrospective validation demonstrated that, in the full study population, the continuous and categorical DS were prognostic for TotBE risk after adjusting for radiotherapy treatment. In the subset of women treated with BCS without radiotherapy, the relative TotBE and InvBE risks for continuous and categorical DS were consistent with prognostic results reported previously for this test (15). Although this study was not designed to assess prediction of radiotherapy benefit described in the earlier study, it marks an important first step in externally validating a promising new test to support clinical decision-making in DCIS.

Disclosure of Potential Conflicts of Interest

S. Weinmann and M.C. Leo reports receiving commercial research grants from Prelude. P.W. Whitworth is a paid advisor and reports receiving commercial research grants from PreludeDx, and reports receiving other commercial research support from TME Research. R. Patel is a paid consultant for TME Consulting. J. Pellicane reports receiving speakers bureau honoraria from and holds ownership interest (including patents) in PreludeDx. F. Wärnberg reports receiving other commercial research support from PreludeDx. T. Bremer is a paid employee of and holds

ownership interest (including patents) in PreludeDx. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Weinmann, C.L. Jenkins, T. Barry

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Weinmann, M.C. Leo, T. Barry, G. Leesman, P.W. Whitworth, R. Patel, F. Wärnberg

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Validation of a Ductal Carcinoma *In Situ* Biomarker Profile for Risk of Recurrence after Breast-Conserving Surgery with and without Radiotherapy

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