Clinical Cancer Research

A Biological Signature for Breast Ductal Carcinoma *In Situ* to Predict Radiotherapy Benefit and Assess Recurrence Risk

Troy Bremer[1], Pat W. Whitworth[2], Rakesh Patel[3], Jess Savala[1], Todd Barry[4], Stephen Lyle[5], Glen Leesman[1], Steven P. Linke[6], Karin Jirström[7], Wenjing Zhou[8], Rose-Marie Amini[9], and Fredrik Wärnberg[10]

Abstract

Purpose: Ductal carcinoma *in situ* (DCIS) patients and their physicians currently face challenging treatment decisions with limited information about the individual's subsequent breast cancer risk or treatment benefit. The DCISionRT biological signature developed in this study provides recurrence risk and predicts radiotherapy (RT) benefit for DCIS patients following breast-conserving surgery (BCS).

Experimental Design: A biological signature that calculates an individualized Decision Score (DS) was developed and cross-validated in 526 DCIS patients treated with BCS ± RT. The relationship was assessed between DS and 10-year risk of invasive breast cancer (IBC) or any ipsilateral breast event (IBE), including IBC or DCIS. RT benefit was evaluated by risk group and as a function of DS.

Results: The DS was significantly associated with IBC and IBE risk, HR (per 5 units) of 4.2 and 3.1, respectively. For patients treated without RT, DS identified a Low Group with 10-year IBC risk of 4% (7% IBE) and an Elevated Risk Group with IBC risk of 15% (23% IBE). In analysis of DS and RT by group, the Elevated Risk Group received significant RT benefit, HR of 0.3 for IBC and IBE. In a clinicopathologically low-risk subset, DS reclassified 42% of patients into the Elevated Risk Group. In an interaction analysis of DS and RT, patients with elevated DS had significant RT benefit over baseline.

Conclusions: The DS was prognostic for risk and predicted RT benefit for DCIS patients. DS identified a clinically meaningful low-risk group and a group with elevated 10-year risks that received substantial RT benefit over baseline. *Clin Cancer Res; 24(23); 5895–901. ©2018 AACR.*

Introduction

Over the last two decades, surgical and adjuvant treatment of ductal carcinoma in situ (DCIS) has advanced significantly. However, surgeons and radiation oncologists, along with their patients, still rely largely on traditional clinical and pathologic risk factors to make treatment decisions. Although these traditional risk factors provide some useful clinical information, they often do not accurately determine a given patient's recurrence risk or—importantly—the clinical benefit of a specific treatment (1–13).

Current treatment of DCIS usually involves breast-conserving surgery (BCS) followed by adjuvant radiotherapy (RT). Mastectomy may also be performed if BCS is not feasible or if elected for other reasons. In a meta-analysis of large prospective clinical trials, patients with DCIS treated with BCS alone had a local recurrence rate of 28% at 10 years for either DCIS or invasive cancer (1). This rate was reduced to 13% in patients who received BCS and RT, which equates to an approximately 50% relative risk reduction. The pooled Early Breast Cancer Trialists Cooperative Group overview concluded that, over 10 years, RT was equally effective regardless of the age at diagnosis, extent of BCS, use of tamoxifen, method of DCIS detection, margin status, presence of comedonecrosis, tumor focality, grade, or architecture (1, 14).

An increased focus on personalized medicine has led to a number of efforts to accurately assess recurrence risk for DCIS patients and predict treatment benefit. However, these efforts all relied upon panels that considered either individual molecular or

[1]PreludeDx, Laguna Hills, California. [2]Nashville Breast Center, Nashville, Tennessee. [3]Good Samaritan Cancer Center, Los Gatos, California. [4]Spectrum Pathology, Inc., Mission Viejo, California. [5]University of Massachusetts Medical School, Worcester, Massachusetts. [6]Steven P. Linke Consulting, Carlsbad, California. [7]Division of Oncology and Pathology, Department of Clinical Sciences, Lund University, Lund, Sweden. [8]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden. [9]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden. [10]Department of Surgical Sciences, Uppsala University, Department of Surgery, Uppsala Academic Hospital, Uppsala, Sweden.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Previous Public Disclosures Related to Data Contained in the Manuscript

Miami Breast Cancer Conference 2016

ASCO 2016

Swedish Surgical Week, Aug 2016

San Antonio Breast Cancer Symposium 2014 (P4-11-17) & (P4-11-18)

San Antonio Breast Cancer Symposium 2011 (P4-18-01) & (P4-10-01)

Wärnberg F, Amini RM, Goldman M, Jirström K. Quality aspects of the tissue microarray technique in a population-based cohort with ductal carcinoma in situ of the breast. *Histopathology*. 2008. doi:10.1111/j.1365-2559.2008.03156.x.

Corresponding Authors: Troy Bremer, PreludeDx, 26051 Merit Circle Suite 102, Laguna Hills, CA 92653. Phone: 949-348-1188; E-mail: tbremer@preludedx.com, and Fredrik Wärnberg, Uppsala Academic, Uppsala, Sweden. E-mail: Fredrik.Warnberg@surgsci.uu.se

doi: 10.1158/1078-0432.CCR-18-0842

Translational Relevance

The clinical dilemma associated with ductal carcinoma *in situ* (DCIS) treatment has been that although survival is excellent for patients, regardless of treatment, subsequent risk of DCIS or invasive breast cancer remains unclear. Further uncertainty surrounds an individual patient's benefit from radiotherapy (RT), one of the most commonly used adjuvant treatments. This uncertainty leads some to make the decision to forego RT, which may result in a future recurrence, whereas others decide to receive the therapy without an individual assessment of benefit. Ultimately, this results from a lack of personalized risk assessment tools capable of distinguishing high-risk patients that would substantially benefit from RT compared with low-risk patients that may receive limited to no clinical benefit. This research presents development and cross-validation of DCISionRT, the first prognostic and predictive test for DCIS. With further clinical evidence, this DCIS biological risk signature has the potential to transform the way DCIS is managed.

clinicopathologic factors that are simply weighted (3, 7, 8, 10, 15). Such linear models are unable to account for molecular interdependencies present in DCIS biology, which adversely effects their performance. This, in addition to limited clinical evidence, has largely prevented their widespread adoption in clinical practice (16–18). Fortunately, advancements in computing power and improved understanding of molecular biology have cleared the way for new development approaches.

When used independently, numerous molecular and clinicopathologic factors have demonstrated promising but variable prognostic results (8, 18). We hypothesized that certain combinations of these factors when assembled appropriately in a nonlinear model would have the power necessary to assess recurrence risk and predict treatment benefit. This idea and the emergence of a limited number of nonlinear terms that were successfully applied to DCIS was the genesis for development of a next-generation risk assessment tool.

Here, we report the development and cross-validation of such a DCIS biological signature called DCISionRT (PreludeDx), addressing the challenge faced in personalizing DCIS treatment.

Materials and Methods

Patient populations

The study was conducted on archived tissue samples in collaboration with Uppsala University Hospital and Västmanland County Hospital, Sweden (UUH), the University of Massachusetts, Worcester (UMass), and PreludeDx (19). Treatment decisions were neither randomized nor strictly rules-based. Patients were included consecutively between 1986 and 2004 at UUH and between 1999 and 2008 at UMass. The study was conducted with the appropriate institutional approvals (see Supplementary Information). The study was conducted in accordance with recognized ethical guidelines and principles from the WMA Declaration of Helsinki for medical research involving human subjects. The study was approved by Uppsala University and UMass Institutional Review Boards and research ethics committees: Uppsala University Regional Ethical Review Board: 1995/170, 1999/422 and 2005:118 and UMass Medical School Tissue and Tumor Bank Institutional Review Board, Institutional Biosafety Committee.

There were 721 female patients diagnosed with a primary DCIS and treated with BCS identified from 2 study sites. Patients were excluded if they had prior breast cancer, simultaneous invasive breast cancer (IBC), or an ipsilateral IBC within 6 months of surgery. The study population is comprised of 526 of these patients who satisfied the inclusion and exclusion criteria, with tissue blocks or tissue microarray biopsies and pathology reports available as well as no missing HER2 or PR markers (Supplementary Fig. S1). Mean follow-up for the study population was 10 years (median, 9 years; range, 0.5–26 years).

DCISionRT biological signature development

A series of literature reviews of publications and patents were conducted to identify molecular markers and clinicopathologic factors associated with DCIS recurrence or progression (8, 14, 20–24). Molecular and clinical factors considered for incorporation into the test were selected from literature reviews as well as from prior unpublished research. The utility of included features (single factors or interactions between and within markers and clinicopathologic factors) was explored using machine learning techniques (see Supplementary Information; refs. 25–28).

Assays were conducted according to standardized protocols adapted for DCIS, which are described in Supplementary Table S1. IBC events included all first IBC events that were ipsilateral (local or regional) or distant metastatic disease. A contralateral invasive breast event prior to a distant metastatic event censored the metastatic IBC event. Total ipsilateral breast events (IBE) included all ipsilateral DCIS events or IBC after the primary DCIS. Analyses were based on time from primary DCIS diagnosis to recurrence. If a patient did not have any subsequent event, censoring occurred at death or last follow-up.

The biological signature, composed of elements identified in Supplementary Fig. S2, was parameterized and tested using multiple cross-validation and produced a consensus continuous risk score on a scale from zero to ten, termed Decision Score (DS). A risk threshold was selected using the training datasets in the cross-validated development with the goal of identifying an average 10-year IBE risk of 10% and an IBC risk of 6% or less. Patients with a score greater than the threshold belonged to the Elevated Risk Group. The threshold between the Low and Elevated Groups was scaled to 3, with the Low Group including patients with DS $\leq$ 3, and the Elevated Risk Group including patients with DS > 3.

Individual factors from the biological signature with the addition of grade were used to construct a linear panel for comparison with the biological signature; for further detail, see Supplementary Materials.

Statistical methods

Summary statistics were generated for clinicopathologic, treatment, and outcome characteristics to compare distributions of patients in the study population and study sites ($n = 526$). Cox proportional hazards regression analysis was summarized as an HR (HR per group or HR per 50% of the range for continuous variables), with 95% confidence interval (CI) and P values. A multivariate Cox regression analysis for relative IBC and IBE risk was performed with covariates for treatment, clinicopathologic factors, and year of diagnosis, and then with DS included. The form of the baseline risk by year of diagnosis, independent of the

DS result, was determined by surveying IBC and IBE risks by the Kaplan–Meier analysis for each diagnosis year (including adjacent ±2 years) over the study period. A multivariate analysis was also performed to assess relative IBC and IBE along with the benefit of RT as a function of DS, adjusting for year of diagnosis. A similar analysis was completed to assess the relative risk of RT conditioned on DS group and study site. This analysis was performed in a subset of the study population that excluded margin-positive patients. A similar analysis was also performed to assess relative risk from DS, and RT benefit by Low and Elevated Risk Groups, adjusting for year of diagnosis. The interaction of RT and DS was further assessed along with DS and RT as independent factors to determine the DS threshold above which there was a significant RT benefit beyond the baseline RT effect. This was done by working backward in the DS range from 3.0 to 1.0. The 10-year absolute risks were calculated using Cox proportional hazards regressions as a function of continuous DS by RT for IBC and IBE risks, adjusted for year of diagnosis after 1995, where monotonic linear or scalar exponential terms were used for DS by RT. A multivariate Cox proportional hazards analysis for relative IBC and IBE risk was also performed with covariates for treatment, clinicopathologic factors, year of diagnosis, and molecular factors.

Results

Patient characteristics of the study population are provided in Table 1, with UUH and UMass study site data. In the study population, 59% of the patients were treated with adjuvant RT, and 29% were treated with adjuvant hormonal treatment (HT). There were minor variations in clinicopathologic factor distribution between patients from the two clinical sites for tumor grade, necrosis, palpability, and age. Fewer patients had positive margins or lesions greater than 1 cm in the UMass patient set. The distribution of the baseline 10-year risks of IBC and total IBE is illustrated in Supplementary Fig. S3 as a function of year of diagnosis. The baseline risk by year of diagnosis indicated a distinct decrease in baseline risk after 1995; therefore, a year of diagnosis threshold was defined as 1996.

A multivariate Cox proportional hazards analysis was performed with clinical, pathologic, and treatment factors and then with the inclusion of the continuous DS calculated by the DCISionRT biological signature (Table 2). The factors, margin status, year of diagnosis (>1995), and RT were significant. When the DS was included, it was significantly correlated with 10-year risks (Table 2). Diagnosis year and RT in patients with elevated DS (DS > 3) also remained significant in the multivariate analysis that included DS. All other clinicopathologic factors were nonsignificant.

A multivariate analysis was performed in the subpopulation of patients that excluded positive margins for DS, where RT benefit was a continuous function of DS, and year of diagnosis was included ($n = 474$). DS was correlated with 10-year IBC and IBE risks and correlated with RT benefit HR (per 5 units) of 4.2 and 3.1 for IBC and IBE, respectively (Table 3). In this study, 196 patients were identified as low risk, of which 112 were treated with RT (57%). There were 278 patients in the Elevated Risk Group, and 166 were treated with RT (60%).

The extent of risk reduction in patients receiving RT was dependent on the DS Group. Patients treated with or without RT had similar outcomes in the Low Group for either IBC (HR, 0.6; $P = 0.485$) or IBE (HR, 0.7; $P = 0.338$; see Table 4). However, the Elevated Risk Group when treated with RT had significantly decreased rates for IBC (HR, 0.3; P, 0.003) and IBE (HR, 0.3; $P < 0.001$). The substantial RT benefit for patients in the Elevated Risk Group was consistent among sites (see Supplementary Table S2). Patients under 50 years of age, with high-grade tumors or tumors larger than 10 mm, were more likely to receive RT, but distribution of these factors was similar for the Low and Elevated Risk Groups (Supplementary Table S3). In an accompanying interaction analysis between DS, RT, and the interaction term RT with DS above a threshold (i.e., RT and DS > X), the interaction term for RT was significant for IBE at DS > 2.7 and at IBC for DS > 2.1, whereas the baseline RT terms were not significant (Supplementary Table S4).

The 10-year absolute risks of IBC and IBE as a function of continuous DS by RT and adjusted for year of diagnosis are provided in Fig. 1. IBC risk significantly increased from 3% to approximately 40% with increasing DS. Similarly, IBE risk significantly increased from 7% to approximately 50% with increasing DS for BCS-treated patients with clear margins.

Table 1. Detailed patient characteristics

Characteristic	UUH ($n = 253$) *n* (%)	UMass ($n = 273$) *n* (%)	Study population ($n = 526$) *n* (%)
Age			
<50	67 (26%)	78 (29%)	145 (28%)
≥50	186 (74%)	195 (71%)	381 (72%)
Size			
<10	88 (35%)	133 (49%)	221 (42%)
≥10	138 (55%)	95 (35%)	233 (44%)
Unknown	27 (11%)	45 (16%)	72 (14%)
Grade			
I (low) or II (intermediate)	137 (54%)	160 (59%)	296 (56%)
III (high)	115 (45%)	93 (34%)	209 (40%)
Unknown	1 (0%)	20 (7%)	21 (4%)
Necrosis			
Absent	65 (26%)	81 (30%)	146 (28%)
Present	81 (32%)	151 (55%)	232 (44%)
Unknown	107 (42%)	41 (15%)	148 (28%)
Margin			
Negative (clear margin)	217 (86%)	257 (94%)	474 (90%)
Positive (involved margin)	36 (14%)	14 (5%)	50 (10%)
Unknown	0 (0%)	2 (1%)	2 (0%)
Detection			
Screening	211 (83%)	229 (84%)	440 (84%)
Clinical	41 (16%)	21 (8%)	62 (12%)
Unknown	1 (0%)	23 (8%)	24 (5%)
Palpable			
No	198 (78%)	229 (84%)	427 (81%)
Yes	53 (21%)	21 (8%)	74 (14%)
Unknown	2 (1%)	23 (8%)	25 (5%)
Diagnosis year			
≤1995	120 (47%)	0 (0%)	120 (23%)
>1995	133 (53%)	273 (100%)	406 (77%)
RT			
No	131 (52%)	85 (31%)	216 (41%)
Yes	122 (48%)	188 (69%)	310 (59%)
Hormone therapy			
No	253 (100%)	118 (43%)	371 (71%)
Yes	0 (0%)	150 (55%)	150 (29%)
Unknown	0 (0%)	5 (2%)	5 (1%)

NOTE: Patient counts and percentages in detailed patient characteristics for UUH and UMass cohorts with combined study populations. Margin status was evaluated as negative for no ink on tumor or positive if there was ink on tumor.

Table 2. Multivariate Cox proportional hazard analysis of treatment and clinicopathologic factors without DS and with DS

	IBC (38 events)		IBE (75 events)	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
Analysis without DS				
Age < 50 years	1.7 (0.8–3.3)	0.153	1.5 (0.9–2.4)	0.132
Tumor size ≥ 10 mm	0.6 (0.3–1.3)	0.193	0.9 (0.6–1.5)	0.709
Palpable lesion	0.8 (0.3–2.5)	0.763	0.8 (0.4–1.8)	0.656
Clinical presentation	1.7 (0.6–4.8)	0.359	1.6 (0.7–3.3)	0.255
High grade	0.9 (0.4–1.8)	0.719	1.3 (0.7–2.1)	0.394
Necrosis	0.7 (0.3–1.6)	0.456	0.8 (0.5–1.5)	0.528
Margin	1.3 (0.5–3.4)	0.579	2.1 (1.2–3.8)	0.015
Year of diagnosis >1995	0.6 (0.3–1.3)	0.205	0.5 (0.3–0.8)	0.006
Endocrine therapy	0.7 (0.2–1.9)	0.451	0.6 (0.3–1.4)	0.244
RT	0.6 (0.3–1.2)	0.128	0.6 (0.3–0.9)	0.034
Analysis with DS				
DS (per 5 units)	3.1 (1.5–6.5)	0.003	1.9 (1.1–3.1)	0.016
RT, low risk (DS ≤ 3)	0.9 (0.3–3.2)	0.918	0.8 (0.3–1.8)	0.555
RT, elevated risk (DS > 3)	0.4 (0.2–0.9)	0.035	0.5 (0.3–0.9)	0.014
Endocrine therapy	0.6 (0.2–1.6)	0.267	0.6 (0.3–1.2)	0.147
Diagnosis year >1995	0.7 (0.4–1.5)	0.396	0.5 (0.3–0.8)	0.008

NOTE: A multivariate Cox proportional hazards analysis was performed with clinical, pathologic, and treatment factors without DS and then with DS in study population ($n = 526$). In the analysis without DS, the full complement of factors is included. In the analysis with DS, only significant factors are shown, except endocrine therapy and RT in low-risk patients (DS ≤ 3), which were not statistically significant and are shown for reference.

The 10-year absolute risks of IBC and IBE for patients with a DS of 3 or less (low risk) had an average 10-year IBC risk of 4% (95% CI, 0%–9%) and IBE risk of 8% (95% CI, 0%–14%), adjusted for year of diagnosis (patients diagnosed after 1995; see Table 5). The difference in 10-year risk between low-risk patients treated with and without RT was 1% or less for IBC and IBE. However, the difference in 10-year risk between Elevated Risk patients treated with and without RT was 6% for IBC and 12% for IBE. These 10-year risk outcomes were nearly identical when assessed by the Kaplan–Meier analysis of patients diagnosed after 1995 (less than 1% absolute differences, data not shown).

In comparison, the 10-year baseline risks (independent of DS) for patients with clear margins treated with RT adjusted for year of diagnosis were 7% (95% CI, 2%–11%) for IBC and 10% (95% CI, 5%–14%) for IBE at 10 years (Table 5). The 10-year total contralateral breast event risk was 8% (95% CI, 5%–12%) for patients treated without adjuvant ipsilateral RT.

The biological signature significantly stratified patients into Low and Elevated Risk Groups, where 41% of patients were identified as low risk. The percentage of Low and Elevated Risk Group patients using standard grade and size criteria is reported in Supplementary Table S5, where DS reclassified greater than 50% of the low- and intermediate-grade patients as elevated risk, and 33% of patients that were grade III or had tumor size >2.5 cm were reclassified as low risk. Similarly, within a low clinicopathologic risk group ($n = 273$) defined as screen detected, tumor size less than 25 mm, low or intermediate grade, and nonpalpable and clear margins, DS reclassified 42% of these patients into the Elevated Risk Group. These reclassified patients had substantial 10-year risks, 23% (95% CI, 7%–36%) IBC and 31% (95% CI, 14%–45%) IBE, when not treated with RT. In contrast, neither age, tumor size, nor tumor grade was able to further stratify patients in the DS Low Group ($P > 0.5$; data not shown).

Table 3. Multivariate analysis of IBC and IBE risks by DS, RT as a function of DS, and year of diagnosis

	IBC (33 events)		IBE (61 events)	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
DS (per 5 units)	4.2 (2.2–8.1)	<0.001	3.1 (1.9–5.0)	<0.001
RT, DS (per 5 units)	0.5 (0.3–0.8)	0.005	0.4 (0.3–0.7)	<0.001
Diagnosis year >1995	0.7 (0.3–1.4)	0.258	0.5 (0.3–0.9)	0.010

NOTE: A multivariate Cox proportional hazards analysis was performed with DS, RT as a function of DS, and year of diagnosis > 1995, excluding patients with positive margins ($n = 474$).

The biological signature substantially improved risk stratification beyond individual clinicopathologic or molecular factors. The utility of individual clinicopathologic and molecular factors was evaluated in a multivariate analysis for 10-year IBE risk, excluding patients with positive margins. Of these factors, only age less than 50 years and RT were significant (Supplementary Fig. S4). Similarly, only age and RT were significant for 10-year IBC risk (data not shown). A linear panel was constructed for comparison with the biological signature. This panel used weighted clinicopathologic and molecular factors to assess recurrence risk, and was able to minimally distinguish a low-prevalence high-risk group but failed to identify a clinically meaningful low-risk group (see Supplementary Table S6).

Discussion

This research presents the development and cross-validation of the DCISionRT biological risk signature. The test calculates a DS reported on a scale from zero to ten, with a threshold of 3.0 identifying the Low and Elevated Risk Groups. The cross-validated results are the first data showing that the DS is prognostic, providing significant stratification of 10-year ipsilateral IBC and total IBE risk with a Low Group having a prevalence of 41%. The results also show, for the first time, a risk classifier that significantly predicts differential RT benefit in Low and Elevated Groups.

With the goal of minimizing recurrences, DCIS patients and their physicians often elect adjuvant RT after BCS because evidence to date has shown all patients—even if clinicopathologically low risk—are expected to receive a 50% relative risk reduction (1, 2, 29). At the same time, some still choose to forgo RT due to the potential side effects, lack of access to radiation oncology facilities, treatment cost, and lack of survival benefit. In either case, better information about a patient's individual biological risk and

Table 4. Multivariate Cox proportional hazard analysis of DS and RT by risk groups

	IBC (33 events)		IBE (61 events)	
(Excludes margin-positive patients)	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
DS (per 5 units)	3.7 (1.8–7.4)	<0.001	2.7 (1.6–4.5)	<0.001
RT, low risk (DS $\leq$ 3)	0.6 (0.2–2.3)	0.485	0.7 (0.3–1.6)	0.338
RT, elevated risk (DS > 3)	0.3 (0.1–0.6)	0.003	0.3 (0.1–0.5)	<0.001
Diagnosis year >1995	0.7 (0.3–1.4)	0.325	0.5 (0.3–0.9)	0.017

NOTE: A multivariate Cox proportional hazards analysis was performed with DS, RT as a function of DS, and year of diagnosis >1995, excluding patients with positive margins ($n = 474$).

therapeutic benefit would enable significantly improved management and treatment of DCIS.

Patients in this study had a 50% relative risk reduction from RT over 10 years as expected (30). This 50% relative risk reduction is maintained even in low-risk groups identified by clinical and pathologic factors. For example, patients in the study population with lesions that are low or intermediate grade, less than 1 cm, and with negative margins had a 10-year IBE risk of 18% after BCS and 9% with BCS and RT. However, patients in the DS Low Group had similar outcomes whether or not they received RT (see Tables 4 and 5). In contrast, patients treated with RT and classified into the Elevated Risk Group received a 70% or greater risk reduction than those without RT (see Table 4). An interaction analysis demonstrated that for patients with elevated DS, there is a statistically greater benefit to RT over baseline (see Supplementary Table S4).

The advances in breast cancer screening and treatment methodologies have contributed to continually improved outcomes for DCIS patients over time as evidenced by numerous observational DCIS studies (9, 19, 15, 31–34). Patient outcomes in the study population follow a similar trend whereby those patients diagnosed prior to 1996 were found to have a significantly higher baseline risk than those diagnosed after 1995 in a multivariate analysis including RT and year of diagnosis as a covariate. Therefore, in order to provide clinically relevant 10-year patient outcomes most representative of patients diagnosed and treated today, absolute risks are provided by adjusting for year of diagnosis.

In our study population, the 10-year baseline risks for patients treated with BCS and RT were 7% and 10% for IBC and IBE, respectively, adjusted for diagnosis after 1995 (see Table 5). This is similar to the rates reported in the United States for DCIS patients in general, treated with BCS and RT (31, 33). In our study population, patients in the Low Group treated without RT had similar average 10-year outcomes of 4% and 8% for IBC and IBE risks which were comparable with the baseline risks for patients treated with BCS and RT. The ability to identify patients with risks as low as those in our Low Group treated with BCS alone will be clinically useful, but clinicians may still prefer to further reduce the remaining risk in the Low Group. However, in our study population, outcomes were similar in the Low Group for patients receiving BCS and BCS with RT (Table 2).

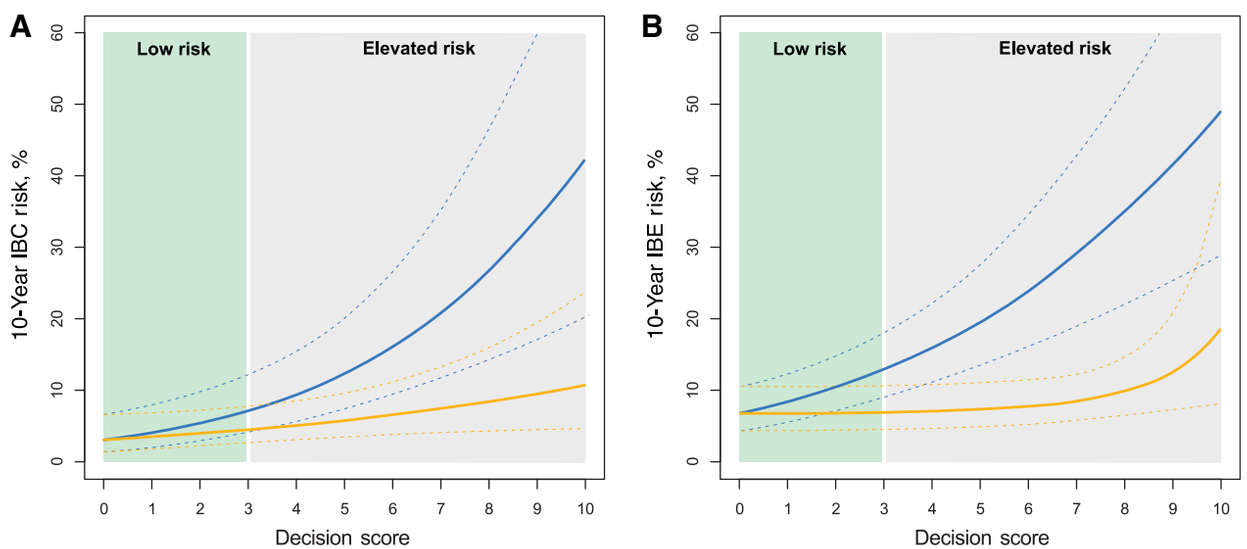

Figure 1.

Continuous risk curves by DS for 10-year IBC and IBE risk, excluding patients with positive margins. **A,** Cox proportional hazards analysis results for the 10-year risk of IBC according to DS by RT adjusted for year of diagnosis in patients with clear margins. The solid blue line represents patients who received BCS alone, and the orange solid line indicates risk for patients who received BCS and RT. The dotted lines represent 95% CIs of the corresponding solid line. The shaded green area denotes the Low Risk Group (DS $\leq$ 3), whereas the shaded gray area represents the Elevated Risk Group (DS > 3). **B,** Cox proportional hazards analysis results for the 10-year risk of IBE according to DS by RT adjusted for year of diagnosis in patients with clear margins. The solid blue line represents patients who received BCS alone, and the orange solid line indicates risk for patients who received BCS and RT. The dotted lines represent 95% CIs of the corresponding solid line. The shaded green area denotes the Low Risk Group (DS $\leq$ 3), whereas the shaded gray area represents the Elevated Risk Group (DS > 3).

Table 5. 10-Year risks for study baseline and DS group

	10-Year risk	
	BCS % (95% CI)	BCS+RT % (95% CI)
IBC		
Study baseline	9 (4–15)	7 (2–11)
Low-risk group (DS $\leq$ 3)	4 (0–9)	3 (0–7)
Elevated-risk group (DS > 3)	15 (5–24)	9 (3–15)
IBE		
Study baseline	15 (8–22)	10 (5–14)
Low-risk group (DS $\leq$ 3)	8 (0–14)	7 (1–13)
Elevated-risk group (DS > 3)	23 (11–33)	11 (4–17)

NOTE: 10-Year ipsilateral risks of IBC and IBE for DS by RT adjusting for year of diagnosis > 1995, by Cox proportional hazards regression analysis, excluding margin-positive patients ($n = 474$).

Because local DCIS management endeavors to eliminate recurrence risk and does not address new primary events, an alternate target of good local control should consider the new primary event rate, which is estimated by the contralateral breast event rate of 8% in this study. Within the Low Group (DS $\leq$ 3), the addition of RT resulted in a 1% or less risk reduction for IBC and IBE, which may be the maximal risk reduction possible from RT. Further risk reduction may be possible with adjuvant (HT).

Determining the patients most likely to progress to IBC is equally important, if not more so, enabling physicians to ensure that patients with a high risk are treated with RT or another appropriate adjuvant therapy. Today, for patients treated with BCS, approximately 20% forgo RT, for any number of reasons but may be at high risk. When presented with evidence that an individual patient's tumor biology is high risk, the decision to forego RT may change. In our study, the DS identified an Elevated Group of patients treated without RT with a high 10-year IBC risk of 15% (23% IBE risk). However, patients in the Elevated Risk Group treated with RT had a 10-year IBC risk of 9% (11% IBE), which are similar outcomes to those patients in the Low Group regardless of treatment. RT resulted in a significant risk reduction for patients in the Elevated Risk Group (6% IBC and 12% IBE).

The findings reflect the effectiveness of the biological signature to identify patients with favorable tumor biology and separate them from the Elevated Risk Group of patients that have the highest likelihood of recurrence. Nonlinear modeling enabled development of this DCIS signature of biological dysregulation and critical oncogenic pathways that integrates combinations of factors. This allows for risk derived from one biomarker to depend on another biomarker, whereas previous development efforts focused linear weighting for each factor, which is unable to account for complex interactions. For example, when the tumor-suppressor protein p16 expression is elevated, cell-cycle progression should be decelerated, as indicated by the cellular proliferation marker Ki-67 having low expression. However, if p16 and Ki-67 expression levels are both elevated, the cell cycle is not being appropriately regulated, leading to increased risk (14, 21).

The treatment in this study population was neither randomized nor strictly rules based. Patients under 50 years of age, with high-grade tumors or tumors larger than 10 mm, were more likely to receive RT (Supplementary Table S3). Although patients not receiving RT may have had a lower baseline risk than those receiving RT, the factors influencing treatment choice (age, size, and grade) did not significantly alter outcomes within the Low Group (data not shown). Further, the DS reclassified 42% of patients with traditionally low-risk clinicopathology (screen detected, tumor size less than 25 mm, low or intermediate grade, nonpalpable, and clear margins) into the Elevated Risk Group, for which the 10-year risks of recurrence were substantial (see Supplementary Information).

The baseline characteristics of the multisite study population were consistent with those demonstrated previously in randomized clinical trials, supporting that this was a suitable population for this development and cross-validation (1). Although it is possible that site-specific treatment could influence patient outcomes, there was no meaningful difference in the degree of RT benefit between study sites for patients in the Elevated Risk Group (Supplementary Table S2).

Further clinical validations are required to increase the level of evidence for the prognostic and predictive capability of the biological signature. Such independent clinical validations in both observational and randomized cohorts are ongoing. With consistent results from subsequent validation, the DCISionRT biological signature will provide an integrated DS that addresses clinical challenges in risk assessment and predicts those patients that benefit most from RT.

Disclosure of Potential Conflicts of Interest

T. Bremer is an employee of and holds ownership interest (including patents) in Prelude Corporation, reports receiving commercial research grants to Prelude Corporation from National Cancer Institute, and is listed as a co-inventor on one or more patents and patent applications licensed to or owned by Prelude Corporation. P.W. Whitworth reports receiving commercial research grants from and is a consultant/advisory board member for PreludeDx, and reports receiving speakers bureau honoraria from PreludeDx and Genomic Health. S.P. Linke is an employee of and holds ownership interest (including patents) in Prelude Corporation. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: T. Bremer, S.P. Linke, F. Wärnberg
Development of methodology: T. Bremer, T. Barry, S.P. Linke
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Savala, T. Barry, S. Lyle, K. Jirström, W. Zhou, R.-M. Amini, F. Wärnberg
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Bremer, P.W. Whitworth, J. Savala, T. Barry, S. Lyle, G. Leesman, S.P. Linke, F. Wärnberg
Writing, review, and/or revision of the manuscript: T. Bremer, P.W. Whitworth, R. Patel, T. Barry, S. Lyle, G. Leesman, S.P. Linke, R.-M. Amini, F. Wärnberg
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Bremer, P.W. Whitworth, S. Lyle, S.P. Linke, K. Jirström, W. Zhou, F. Wärnberg
Study supervision: S. Lyle, S.P. Linke, F. Wärnberg

Acknowledgments

Each site received funding from the sponsor, PreludeDx, to cover the cost of collecting tissue specimens and performing data collection. Analysis and interpretation of data and preparation, review, approval of the article, and decision to submit were mutually agreed upon among the principal investigators and the sponsor.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 16, 2018; revised June 19, 2018; accepted July 25, 2018; published first July 27, 2018.

References

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Correa C, McGale P, Taylor C, Wang Y, Clarke M, et al. Overview of the randomized trials of radiotherapy in ductal carcinoma in situ of the breast. J Natl Cancer Inst - Monogr 2010;2010:162–77.
2. EORTC Breast Cancer Cooperative Group; EORTC Radiotherapy Group, Bijker N, Meijnen P, Peterse JL, Bogaerts J, et al. Breast-conserving treatment with or without radiotherapy in ductal carcinoma-in-situ: ten-year results of european organisation for research and treatment of cancer randomized phase III trial 10853—a study by the EORTC breast cancer cooperative group and EORTC radiotherapy group. J Clin Oncol 2006;24:3381–7.
3. Rakovitch E, Nofech-Mozes S, Hanna W, Sutradhar R, Baehner FL, Miller DP, et al. Multigene expression assay and benefit of radiotherapy after breast conservation in ductal carcinoma in situ. J Natl Cancer Inst 2017;109:djw256.
4. Rakovitch E, Nofech-Mozes S, Hanna W, Baehner FL, Saskin R, Butler SM, et al. A population-based validation study of the DCIS Score predicting recurrence risk in individuals treated by breast-conserving surgery alone. Breast Cancer Res Treat 2015;152:389–98.
5. Thorat MA, Parmar V, Nadkarni MS, Badwe RA. Radiation therapy for ductal carcinoma in situ: is it really worth it? J Clin Oncol 2007;25:461–2; author reply 462.
6. Punglia RS, Burstein HJ, Weeks JC. Radiation therapy for ductal carcinoma in situ: a decision analysis. Cancer 2012;118:603–11.
7. Rudloff U, Jacks LM, Goldberg JI, Wynveen CA, Brogi E, Patil S, et al. Nomogram for predicting the risk of local recurrence after breast-conserving surgery for ductal carcinoma in situ. J Clin Oncol 2010;28:3762–9.
8. Lari SA, Kuerer HM. Biological markers in DCIS and risk of breast recurrence: a systematic review. J Cancer 2011;2:232–61.
9. Di Saverio S, Catena F, Santini D, Ansaloni L, Fogacci T, Mignani S, et al. 259 Patients with DCIS of the breast applying USC/Van Nuys prognostic index: a retrospective review with long term follow up. Breast Cancer Res Treat 2008;109:405–16.
10. Silverstein MJ, Lagios MD, Craig PH, Waisman JR, Lewinsky BS, Colburn WJ, et al. A prognostic index for ductal carcinoma in situ of the breast. Cancer 1996;77:2267–74.
11. MacAusland SG, Hepel JT, Chong FK, Galper SL, Gass JS, Ruthazer R, et al. An attempt to independently verify the utility of the van nuys prognostic index for ductal carcinoma in situ. Cancer 2007;110:2648–53.
12. Gilleard O, Goodman A, Cooper M, Davies M, Dunn J. The significance of the Van Nuys prognostic index in the management of ductal carcinoma in situ. World J Surg Oncol 2008;6:61.
13. Wong JS, Chen YH, Gadd MA, Gelman R, Lester SC, Schnitt SJ, et al. Eight-year update of a prospective study of wide excision alone for small low- or intermediate-grade ductal carcinoma in situ (DCIS). Breast Cancer Res Treat 2014;143:343–50.
14. Kerlikowske K, Molinaro AM, Gauthier ML, Berman HK, Waldman F, Bennington J, et al. Biomarker expression and risk of subsequent tumors after initial ductal carcinoma in situ diagnosis. J Natl Cancer Inst 2010;102:627–37.
15. Solin LJ, Gray R, Hughes LL, Wood WC, Lowen MA, Badve SS, et al. Surgical excision without radiation for ductal carcinoma in situ of the breast: 12-year results from the ECOG-ACRIN E5194 study. J Clin Oncol 2015; 33:3938–44.
16. Yi M, Meric-Bernstam F, Kuerer HM, Mittendorf EA, Bedrosian I, Lucci A, et al. Evaluation of a breast cancer nomogram for predicting risk of ipsilateral breast tumor recurrences in patients with ductal carcinoma in situ after local excision. J Clin Oncol 2012;30:600–7.
17. Javid SH, Anderson BO. Tailored strategies for DCIS management. Oncology 2011;25:861–3.
18. Gorringe KL, Fox SB. Ductal carcinoma in situ biology, biomarkers, and diagnosis. Front Oncol 2017;7:248.
19. Zhou W, Jirström K, Johansson C, Amini RM, Blomqvist C, Agbaje O, et al. Long-term survival of women with basal-like ductal carcinoma in situ of the breast: a population-based cohort study. BMC Cancer 2010;10:653.
20. Witkiewicz AK, Dasgupta A, Nguyen K, Liu C, Kovatich AJ, Schwartz GF, et al. Cancer biology & therapy stromal caveolin-1 levels predict early DCIS progression to invasive breast cancer. Cancer Biol Ther 2009;8:1071–9.
21. Gauthier ML, Berman HK, Miller C, Kozakeiwicz K, Chew K, Moore D, et al. Abrogated response to cellular stress identifies DCIS associated with subsequent tumor events and defines basal-like breast tumors. Cancer Cell 2007;12:479–91.
22. Lu J, Guo H, Treekitkarnmongkol W, Li P, Zhang J, Shi B, et al. 14-3-3 cooperates with ErbB2 to promote ductal carcinoma in situ progression to invasive breast cancer by inducing epithelial-mesenchymal transition. Cancer Cell 2009;16:195–207.
23. Witkiewicz AK, Freydin B, Chervoneva I, Potoczek M, Rizzo W, Rui H, et al. Stromal CD10 and SPARC expression in ductal carcinoma in situ (DCIS) patients predicts disease recurrence. Cancer Biol Ther 2010; 10:391–6.
24. Makretsov NA, Hayes M, Carter BA, Dabiri S, Gilks CB, Huntsman DG. Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade. Mod Pathol 2007;20:84–9.
25. Picard RR, Cook RD. Cross-validation of regression models. J Am Stat Assoc 1984;79:575–83.
26. Käll L, Storey JD, Noble WS. qvality: non-parametric estimation of q-values and posterior error probabilities. Bioinformatics 2009;25:964–6.
27. Storey JD. A direct approach to false discovery rates. J R Stat Soc Ser B (Statistical Methodol) 2002;64:479–498.
28. Kohavi R. A study of cross-validation and bootstrap for accuracy estimation and model selection. Appear Int Jt Conf Articial Intell 1995:1–7.
29. Wärnberg F, Garmo H, Emdin S, Hedberg V, Adwall L, Sandelin K, et al. Effect of radiotherapy after breast-conserving surgery for ductal carcinoma in situ: 20 years follow-up in the randomized SweDCIS trial. J Clin Oncol 2014;32:3613–8.
30. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. JAMA Oncol 2015;1: 888–96.
31. McCormick B, Winter K, Hudis C, Kuerer HM, Rakovitch E, Smith BL, et al. RTOG 9804: a prospective randomized trial for good-risk ductal carcinoma in situ comparing radiotherapy with observation. J Clin Oncol 2015;33: 709–15.
32. Abe O, Abe R, Enomoto K, Davies C, Elphinstone P, Evans V, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;366:2087–106.
33. Wapnir IL, Dignam JJ, Fisher B, Mamounas EP, Anderson SJ, Julian TB, et al. Long-term outcomes of invasive ipsilateral breast tumor recurrences after lumpectomy in NSABP B-17 and B-24 randomized clinical trials for DCIS. J Natl Cancer Inst 2011;103:478–88.
34. Cuzick J, Sestak I, Pinder SE, Ellis IO, Forsyth S, Bundred NJ, et al. Effect of tamoxifen and radiotherapy in women with locally excised ductal carcinoma in situ: long-term results from the UK/ANZ DCIS trial. Lancet Oncol 2011;12:21–9.