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CLINICAL INVESTIGATION

A Novel Biosignature Identifies Patients With DCIS With High Risk of Local Recurrence After Breast Conserving Surgery and Radiation Therapy

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Purpose: There is an unmet need to identify women diagnosed with ductal carcinoma in situ (DCIS) with a low risk of in-breast recurrence (IBR) after breast conserving surgery (BCS), which could omit radiation therapy (RT), and also to identify those with elevated IBR risk remaining after BCS plus RT. We evaluated a novel biosignature for a residual risk subtype (RRt) to help identify patients with elevated IBR risk after BCS plus RT.

Methods and Materials: Women with DCIS treated with BCS with or without RT at centers in the US, Australia, and Sweden (n = 926) were evaluated. Patients were classified into 3 biosignature risk groups using the decision score (DS) and the RRt

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category: (1) Low Risk (DS ≤2.8 without RRt), (2) Elevated Risk (DS >2.8 without RRt), and (3) Residual Risk (DS >2.8 with RRt). Total and invasive IBR rates were assessed by risk group and treatment.

Results: In patients at low risk, there was no significant difference in IBR rates with or without RT (total, $P = .8$; invasive IBR, $P = .7$), and there were low overall 10-year rates (total, 5.1%; invasive, 2.7%). In patients with elevated risk, IBR rates were decreased with RT (total: hazard ratio [HR], 0.25; $P < .001$; invasive: HR, 0.28; $P = .005$); 10-year rates were 20.6% versus 4.9% (total) and 10.9% versus 3.1% (invasive). In patients with residual risk, although IBR rates decreased with RT after BCS (total: HR, 0.21; $P < .001$; invasive: HR, 0.29; $P = .028$), IBR rates remained significantly higher after RT compared with patients with elevated risk (HR, 2.5; 95% CI, 1.2-5.4; $P = .018$), with 10-year rates of 42.1% versus 14.7% (total) and 18.3% versus 6.5% (invasive).

Conclusions: The novel biosignature identified patients with 3 distinct risk profiles: Low Risk patients with a low recurrence risk with or without adjuvant RT, Elevated Risk patients with excellent outcomes after BCS plus RT, and Residual Risk patients with an elevated recurrence risk remaining after BCS plus RT, warranting potential intensified or alternative treatment approaches.

Introduction

Ductal carcinoma in situ (DCIS) represents approximately 20% of all breast cancers diagnosed in the US each year.[1] Breast conserving surgery (BCS) followed by adjuvant radiation therapy (RT) has remained the standard of care for most patients with DCIS; 4 randomized studies assessing the value of RT after lumpectomy demonstrated consistent relative reduction in local recurrences (40%-50%) compared with BCS alone.[2,3] Presently, the assumption is that all patients with DCIS derive a relatively consistent benefit from adjuvant RT. As such, research has focused on an attempt to identify patients who have a sufficiently low absolute risk of recurrence without RT, such that it would be reasonable to avoid the toxic effects, cost, and inconvenience of RT. Unfortunately, even when selecting DCIS patients with perceived favorable clinicopathologic features (such as nuclear grade 1-2 [low and intermediate] tumor or a smaller tumor upto 2.5 cm), multiple contemporary studies have demonstrated elevated risks of local recurrence when omitting RT (>10% at 10 years) and have failed to identify a subset of patients who do not derive a clinically meaningful benefit from RT with respect to local control.[4-7]

Prognostic assays such as the Van Nuys Prognostic Index and DCIS nomograms estimate the risk of recurrence, but they do not predict RT benefit in patients with DCIS.[8] Owing to the inability of traditional clinicopathologic features to adequately identify low-risk patients with DCIS or predict RT benefit, recent studies have focused on developing a biosignature based on individual patient tumor biology to help identify patients with low-risk and elevated-risk DCIS with respect to long-term outcomes.[9] This approach allows for patient-specific risk stratification such that patients with low-risk DCIS may consider omission of RT, whereas higher-risk patients may consider RT as well as further intensification of treatment, including elevated radiation doses (eg, boost) and/or systemic therapy approaches.[7,8] Examples of biosignatures for DCIS include the OncotypeDx DCIS[10] and the 7-gene predictive DCIS assay, DCISionRT.[11] The OncotypeDx DCIS score estimates the risk of local recurrence after DCIS but does not report RT benefit. In contrast, DCISionRT is a clinical-genomic biosignature that is prognostic for recurrence risk after BCS and predictive for RT benefit.[11-14] The DCISionRT test reports a "decision score" (DS) based on a biosignature that combines the biomarkers and clinicopathologic factors.[11] The DS biosignature accounts for interactions between the different biomarkers and the clinicopathologic factors using a nonlinear algorithm such that the coefficient for a given risk factor depends on the values of other risk factors. However, previous development efforts that were focused on prognostic or predictive tests for breast cancer used linear weighting for each biomarker,[8,10] which did not account for these complex interactions. The DCISionRT biosignature accounts for the interdependencies and activation of the oncogenic pathways commonly dysregulated in DCIS, such as the estrogen response pathway, HER2 pathway, as well as cell cycle, survival, and stress response, leading to increased proliferation and cell survival.

The DCISionRT test was developed to help address the questions of which patients will have clinically low risk with minimal RT benefit and which patients will have more elevated risk with meaningful RT benefit. However, results from prior studies also indicated that there is a subset of the patients with elevated DS results who have a higher risk of recurrence after BCS and RT[11] than traditionally seen. Given that biological features contribute significantly to the differences in outcomes and progression of disease,[15,16] it was hypothesized that the biology underlying these patients was different than that of other patients with DCIS and some specific pathways were driving the aggressiveness and therefore recurrence risk. Genomic studies have revealed that DCIS shares similar genomic heterogeneity to invasive breast cancer, comprising lesions that vary in their clinical presentation and outcomes. Thus, to further identify the subset of the patients with a greater risk of recurrence after BCS and RT, we searched for additional pathways regulated by the existing DCISionRT biomarkers that had a large effect on progression of breast cancer and contributed to the resistance of standard therapies.

Activation of the EGFR/HER2/KRAS pathway has been recently shown to be associated with more aggressive tumor

phenotypes and resistance to standard therapies.[17,18] Studies have shown that key biomarkers included in the validated DS biosignature regulate activity of the EGFR/HER2/KRAS pathway in breast cancer. Moreover, one of the markers (SIAH2) has been recently implicated as the terminal gatekeeper of the EGFR/HER2/KRAS pathway,[19] playing a key role in KRAS-dependent tumor progression. Thus, an algorithm was prespecified to combine biomarkers (used by the DS biosignature) in a novel manner (distinct from the original DS biosignature) based on the biologic hypothesis that an activated EGFR/HER2/KRAS pathway would drive a proliferative, aggressive disease profile and thus could identify a subgroup of patients with higher residual risk after adjuvant RT. It was hypothesized that a test that integrates the DS biosignature with this novel biosignature would identify a subgroup of patients with a high risk of recurrence after BCS and a worse-than-expected outcome after treatment with BCS-adjuvant RT—ie, a residual risk subtype (RRt) group.

Therefore, we sought to validate the utility of the DS and RRt biosignatures integrated into the DCISionRT test to assess the long-term outcomes of adjuvant RT after BCS in a modern cohort of patients with DCIS treated with BCS with or without RT. The test was expected to classify patients into 3 distinct risk populations: (1) those with a low 10-year recurrence risk with or without adjuvant RT and deriving no significant benefit from adjuvant RT (Low Risk group), (2) those with an elevated 10-year recurrence risk without RT who may benefit substantially from RT (Elevated Risk group), and (3) those with an elevated 10-year recurrence risk remaining after RT and who may benefit from intensified or alternative treatment approaches (Residual Risk group). The study examined outcomes in a large group of patients with DCIS treated with BCS with and without RT, evaluating the ability of this integrated biosignature to predict IBR risk and adjuvant RT treatment benefit for patients in these 3 risk populations.

Methods and Materials

Biosignature development

The RRt biosignature reports a binary categorial result that was integrated with the continuous DS biosignature into the DCISionRT test. The DS biosignature combined information from 7 protein tumor biomarkers (COX-2, FOXA1, HER2, Ki-67, p16/INK4A, PR, and SIAH2) and 4 clinicopathologic factors (age at diagnosis, tumor size, palpability, and surgical margin status). The DS biosignature result alone was previously cross-validated in cohorts from Uppsala University Hospital and Västmanland County Hospital, Sweden (UUH), (patients recruited between 1986 and 2004), University of Massachusetts, Worcester (UMASS),[11] (patients recruited between 1999 and 2008), and independently validated in patients recruited between 1990 to 2007 at Kaiser Permanente Northwest (KPNW)[12] and between 2006 and 2011 at The Royal Melbourne Hospital and Royal Women's Hospital, Parkville, Victoria, Australia (RMH).[14] Of note, the Residual Risk subtype was not previously evaluated in these 4 patient cohorts. The DS biosignature was further validated in a randomized clinical trial cohort (SweDCIS)[13] that also defined 2 categorical risk groups: (1) a Low Risk (DS $\leq$ 2.8) group with minimal to no benefit from RT and (2) an Elevated Risk (DS $>$ 2.8) group with a statistically significant reduction from RT and a significant multiplicative interaction between RT and DS.[11,13,14] Thus, the novel prespecified residual-risk subtype was used with the validated continuous DS biosignature result and a threshold of DS = 2.8 to define 3 categorical risk groups as follows: (1) Low Risk group (DS $\leq$ 2.8 without RRt), (2) Elevated Risk group (DS $>$ 2.8 without RRt), or (3) Residual Risk group (DS $>$ 2.8 with RRt).

Study design

An in-breast recurrence (IBR) was defined as either a subsequent ipsilateral local or regional DCIS or invasive breast cancer diagnosis after the primary ipsilateral DCIS diagnosis, excluding metastatic events. Analyses were based on the time to the first IBR at least 6 months after the primary surgery. A contralateral breast recurrence (CBR) was defined as a subsequent CBR event (either DCIS or invasive cancer).

The objectives were to assess the association of the 3 categorical biosignature risk groups with the IBR rate after treatment with BCS and RT, to assess the association of RT with IBR rates within categorical biosignature risk groups, and to assess the association of categorical biosignature risk groups with IBR rates after treatment with BCS without RT. Other planned analyses assessed the multiplicative interaction of RT and biosignature risk groups, the utility of the biosignature risk groups accounting for clinicopathologic factors, and the association of continuous DS with the IBR rate.

Patients and sample preparation

Patients were treated with BCS with or without adjuvant RT therapy and optionally with hormone therapy at UUH and UMASS[11] and at KPNW[12] and RMH.[14] Treatment decisions were neither randomized nor strictly rules-based. Patients were excluded if they had a prior breast cancer or a simultaneous invasive breast cancer. All evaluable patients were treated with BCS with negative margins and complete biomarker data. The testing was performed, blinded to outcome, on intact formalin fixed paraffin embedded tissue mounted slides, which preserved tissue architecture. This enabled the protein expression to be evaluated only in epithelial DCIS tissue while excluding contaminating effects from other tissue. All the clinicopathologic parameters used in the study were defined based on previous DCISionRT studies.[11-14] Pathology data were obtained from pathology reports augmented by central pathology review. Clinical data were obtained from electronic and/or paper medical records. For quality assurance purposes, a subset of patient records was reabstracted

and reviewed for accuracy at each of the sites. The study was conducted in accordance with recognized ethical guidelines and principles from the Declaration of Helsinki for medical research involving human subjects. The study was approved by ethics committees for UUH[11] and RMH,[14] and institutional review boards for UMASS[11] and KPNW.[12]

Statistical analyses

Kaplan-Meier analysis was used to compute IBR curves and average 10-year total IBR and invasive IBR rates and 95% confidence intervals (CIs) for categorical biosignature risk groups. Log-rank testing was used to assess the differences in IBR rates between different categorical biosignature risk groups; the association of the Elevated and Residual Risk groups with IBR rate relative to the Low Risk group in patients treated with BCS without RT and the association of the Residual Risk group with IBR rate relative to the Elevated Risk and combined Low- and Elevated Risk groups in patients treated with BCS plus RT were determined by univariate Cox proportional hazards analysis during all follow-up and a period of 0 to 10 years, because new primary ipsilateral breast events are expected to occur during all follow-up and because ipsilateral breast recurrences from the diagnosed DCIS tumor were expected during the 0-to-10-year period. The utility of the biosignature risk groups with RT to predict IBR rate after controlling for clinicopathologic factors and endocrine treatment was tested using multivariable Cox proportional hazards analysis. The interaction between biosignature categorical risk groups and RT benefit was also tested by comparing a model including a term for the interaction of treatment and risk group with a model including only main effects. Because the biosignature risk groups were determined with an algorithm that combined biomarkers and clinicopathologic factors, the utility of the biosignature risk groups to predict the IBR rate compared with treatment and standard clinicopathologic factors alone was tested using the likelihood ratio, comparing a model that included the biosignature risk groups, treatment−risk group interaction, and clinicopathologic factor terms with a model that included only clinicopathologic factor and treatment terms. The association between continuous DS (linear) with IBR rate was also assessed by RT treatment in all evaluable patients and after excluding those in the Residual Risk group. Differences in the distribution of clinicopathologic factors between radiation treatment groups or between subsets of patient cohorts were evaluated using the χ^2 test. Analyses were performed for all evaluable patients from the 4 cohorts because none of these patients was previously used to evaluate the residual-risk subtype, and analyses were performed separately for the subset of patients from RMH and KPNW cohorts, which were previously used to independently validate the DS biosignature. The log-rank test and Cox proportional hazards analysis with the Wald test were used to assess the differences in IBR rates between the RMH/KPNW and UUH/UMASS patient subsets within biosignature risk groups and by treatment over all follow-up. All inferential tests were evaluated using a 2-tailed α level of .05. Statistical analyses were performed by an independent statistical analysis group (McCloud Consulting Group). Study results are reported consistent with Reporting Recommendations for Tumor Marker Prognostic Studies guidelines.

Results

There were 926 evaluable patients from the 4 combined cohorts (Fig. E1 [Reporting Recommendations for Tumor Marker Prognostic Studies diagram]). Patients treated with RT were more likely to be under 50 years of age, have tumors of a larger size, or have a higher nuclear grade (Table 1). A total of 316 patients (34%) were prescribed hormone therapy (ET), of which 232 (73%) also received RT. Mean follow-up for the evaluable study population was 8.8 years (median, 8.5 years; 1st-3rd quartile, 5.8-10.2 years), with a total of 92 events recorded overall. There were 41 DCIS and 36 invasive events in the interval from 0 to 10 years and 3 DCIS and 12 invasive events after 10 years (Table 1).

The distribution of clinicopathologic features and the number of in-breast events was summarized by biosignature categorical risk groups (Table E1). There were 338 patients (37%) classified into the Low Risk group (DS $\leq$ 2.8 without RRt), 399 (43%) classified into the Elevated- Risk group (DS$\leq$2.8 without RRt), and 189 (20%) classified into the Residual- Risk group (DS > 2.8 with RRt). Patients in the Residual- Risk group had a higher rate of nuclear grade 3 DCIS, larger tumors (>1 cm in size), and HER2(3+) disease. In patients with HER2(3+) disease (n = 133), 55% were in the Residual- Risk group, whereas 45% were in the Low- and Elevated- Risk groups. Similarly, 40% of the patients with nuclear grade 3 disease were in the Residual- Risk group. There were no other noted differences in clinicopathologic features or adjuvant endocrine treatment between the Residual Risk group and the other risk groups.

Clinicopathologic characteristics were also provided by cohort (Table E2), and the evaluable patients from the RMH and KPNW cohorts were provided separately (Table E3). Of these 593 evaluable patients treated with BCS with negative margins from the RMH and KPNWcohorts, 230 patients (39%) were classified into the Low Risk group, 242 (41%) into the Elevated Risk group, and 121 (20%) into the Residual Risk group. Mean follow-up for the RMH and KPNW study population was 9.6 years (median, 8.9 years; 1st-3rd quartile, 5.8-12.6 years), with 25 DCIS and 20 invasive events in the interval from 0 to 10 years and 3 DCIS and 12 invasive events after 10 years.

Residual Risk group (DS > 2.8 with RRt), IBR rates, and RT benefit

Among all evaluable patients treated with BCS without RT, the IBR rate was elevated for those in the Residual Risk

Table 1 Distribution of clinicopathologic and hormone therapy treatment by radiation therapy treatment for all evaluable patients

Characteristic	BCS plus RT treatment (n = 641), n (%)	BCS without RT treatment (n = 285), n (%)	*P* value*
Age, y			
<50	162 (25.3)	48 (16.8)	.004
≥50	479 (74.7)	237 (83.2)	
Tumor size, mm			
≤10	417 (65.1)	208 (73.0)	.02
>10	224 (34.9)	77 (27.0)	
Margin status			
Free margin	641 (100)	285 (100)	-
Tumor palpable			
No	577 (91.6)	239 (86.3)	.02
Yes	53 (8.4)	38 (13.7)	
Nuclear grade			
1 or 2	374 (58.3)	201 (70.5)	<.001
3	267 (41.7)	84 (29.5)	
Hormone therapy			
Yes	232 (36.4)	84 (29.5)	.04
No	406 (63.6)	201 (70.5)	
Year of diagnosis			
<1996	89 (13.9)	51 (17.9)	.12
≥1996	552 (86.1)	234 (82.1)	
IBR events (0-10 years)			
DCIS or invasive	36 (5.6)	41 (14.4)	-
Invasive	18 (2.8)	18 (7.4)	
IBR events (overall)			
DCIS or invasive	49 (7.6)	43 (15.1)	-
Invasive	28 (4.4)	20 (7.0)	

Abbreviations: BCS = breast conserving surgery; DCIS = ductal carcinoma in situ; IBR = in-breast recurrence; RT = radiation therapy.
* χ^2 test, excluding patients with missing or unknown responses.

group, in which the 10-year total IBR rate was 42.1% (95% CI, 25.9%-63.0%) (Fig. 1), whereas the invasive IBR rate was 18.3% (95% CI, 7.6%-40.1%) (Table E4). Patients in the Residual- Risk group had higher IBR rates compared with other risk categories (total $P_{\text{logrank}} < .001$; invasive $P_{\text{logrank}} = .02$), and the total IBR events occurred sooner in patients in the Residual- Risk group than in the Low- Risk or Elevated- Risk groups (Fig. E2A).

Within the Residual Risk group, those treated with RT had a lower IBR rate (total $P_{\text{logrank}} < .001$, invasive $P_{\text{logrank}} = .02$) compared with those treated without adjuvant RT (Table E4 and Fig. E3A). There was a corresponding relative total IBR rate reduction from RT in the 0-to-10-year interval (hazard ratio [HR], 0.20; 95% CI, 0.10-0.42) (Table 2) and a reduction in the invasive IBR rate from RT (HR, 0.12; 95% CI, 0.02-0.66) (Table E4). However, patients in the Residual Risk group treated with BCS plus RT had a higher total IBR rate compared with other risk categories (ie, Low- or Elevated Risk groups treated with BCS plus RT) in the 0-to-10-year period (HR, 2.5; 95% CI, 1.3-5.0; $P = .005$) or compared with patients in the Elevated Risk group treated with BCS plus RT in the 0-to-10-year period (HR, 2.5; 95% CI, 1.2-5.4; $P = .018$) (Table E5). Patients treated with BCS plus RT tended to have higher invasive IBR rates in the Residual Risk group than in other risk categories (HR, 2.3; 95% CI, 0.87-5.8; $P = .08$) (Table E5).

An assessment of the IBR rates and biosignature risk groups was also performed in the subset of 593 patients from the RMH/KPNWcohorts. There was not a statistically significant difference in the IBR rates for patients in the Residual Risk group between the RMH/KPNW and the UUH/UMASS cohorts overall (total IBR $P_{\text{logrank}} = .63$;

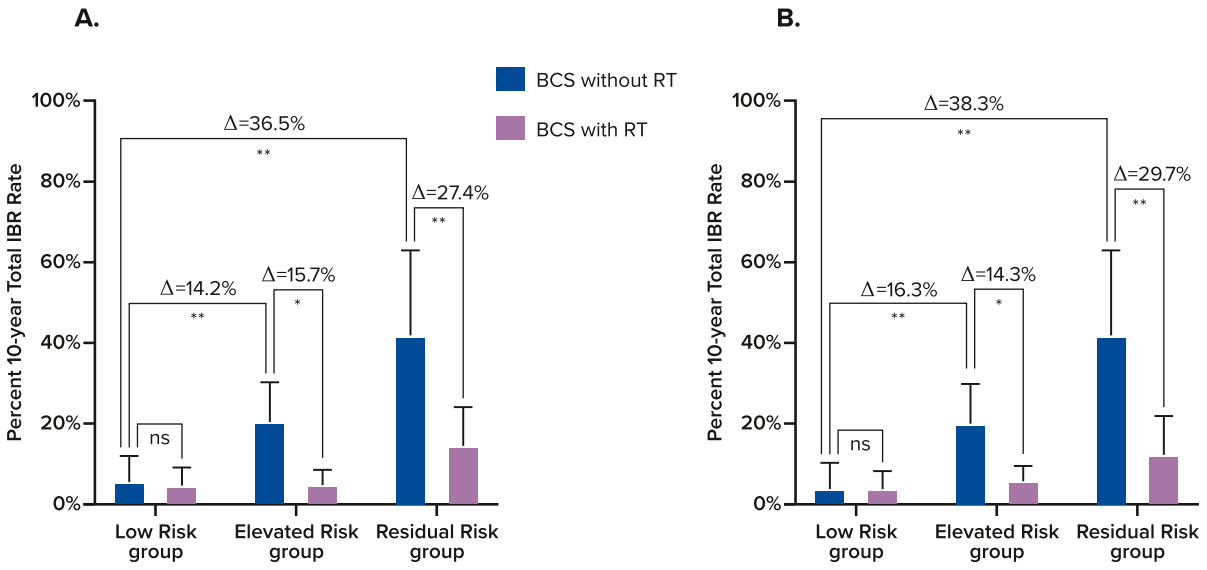

Fig. 1. Ten-year in-breast recurrence (IBR) rates after breast conserving surgery (BCS) by radiation therapy (RT) treatment and by biosignature risk group. Rates of IBR 10 years after treatment with BCS plus RT or BCS without RT by biosignature risk groups. Biosignature risk groups (defined by decision score [DS] and residual risk subtype [RRt]): Low- Risk group (DS < 2.8, without RRt), Elevated- Risk group (DS >2 .8 without RRt), and Residual Risk group (DS > 2.8 with RRt). (A) All evaluable patients (n = 926). (B) RMH/KPNW study cohorts (n = 593). *$P < .05$; **$P < .001$; ns = not significant.

invasive $P_{\text{logrank}} = .24$) (Table E6). Among the patients in the RMH/KPNW cohort treated without RT, those in the Residual Risk group had a higher IBR rate compared with those in the Low Risk group (Table E7). Likewise, those in the Residual Risk group had a higher total IBR rate compared with the Elevated- or Low Risk group over the 0-to-10-year period (total HR, 4.8; 95% CI, 2.0-11.1). Patients treated with BCS plus RT had a lower total IBR rate compared with those treated without RT in the 0-to-10-year interval (total HR, 0.16; 95% CI, 0.06-0.42) (Table 2) and similarly for invasive IBR rates (HR, 0.12; 95% CI, 0.02-0.66) (Table E7 for all follow-up).

Low Risk group (DS ≤ 2.8 without RRt) IBR rates and RT benefit

Among evaluable patients treated with and without RT, those in the Low Risk group had a clinically low IBR rate, where the 10-year total IBR rate was 5.1% (3.1%-8.5%) (Fig. 1) and the 10-year invasive IBR rate was 2.7% (1.2%-5.8%) (Table E4). Those treated with adjuvant RT after BCS did not have a statistically significantly different absolute IBR rate than those treated without RT (total $P_{\text{logrank}} = .78$; invasive $P_{\text{logrank}} = .66$) (Table E4 and Fig. E3A), where the difference in the 10-year total IBR rate was Δ = 0.8 (95% CI,

Table 2 Relative rate reduction in IBR from radiation therapy treatment by biosignature risk groups*

	Total IBR relative RT risk reduction in all evaluable patients†			Total IBR relative RT risk reduction in RMH/KPNW study cohorts‡		
Risk group	n (%) (926 patients, 77 events)	HR (95% CI)§	*P* value	n (%) (593 patients, 45 events)	HR (95% CI)§	*P* value
Low Risk	338 (37)	0.82 (0.29-2.3)	.71	230 (39)	0.81 (0.19-3.4)	.78
Elevated Risk	399 (43)	0.23 (0.11-0.47)	<.001	242 (41)	0.28 (0.11-0.69)	.006
Residual Risk	189 (20)	0.20 (0.10-0.42)	<.001	121 (20)	0.16 (0.06-0.42)	<.001

Abbreviations: HR = hazard ratio; IBR = in-breast recurrence; RT = radiation therapy.

* Relative IBR rate reduction for RT treatment by biosignature risk group over an interval of 0-10 years.

† Total IBR relative risk reduction for RT among all evaluable patients.

‡ Total IBR relative risk reduction for RT among the RMH/KPNW study cohorts.

§ Cox proportional hazards analysis for patients treated with breast-conserving surgery (BCS) plus RT compared with BCS without RT within biosignature risk groups. *P* values are from the Wald test. Biosignature risk categories: LowRisk group (decision score [DS] <2.8, without residual risk subtype [RRt]), Elevated Risk group (DS >2.8 without RRt), and Residual Risk group (DS >2.8 with RRt).

−4.6% to 6.2%) with RT versus without RT (Table E4A). For patients treated without RT, the total IBR rate was not significantly different than the contralateral breast event rate: IBR, 5.6% (2.5%-12.1%) vs CBR: 4.1% (2.0%-8.5%).

There was not a statistically significant difference in the IBR rates for evaluable patients in the Low Risk group between those from the RMH/KPNW subset or the UUH/UMASS subset overall (total IBR $P_{\text{logrank}} = .18$; invasive $P_{\text{logrank}} = .48$) (Table E6). Evaluable patients from the RMH/KPNW cohort in the Low Risk group had a clinically low IBR rate, with an overall 10-year total IBR rate of 3.9% (95% CI, 1.3%-11.7%) (Fig. 1B). Those treated with BCS plus RT did not have a statistically significant different IBR rate than patients treated without RT (total $P_{\text{logrank}} = .86$ invasive: $P_{\text{logrank}} = 0.89$) (Table E8). The corresponding absolute difference at 10 years in the total IBR rate was $\Delta = 0.0\%$ (95% CI, −5.5% to 5.6%), and in the invasive IBR rate it was $\Delta = -1.3\%$ (95% CI, −5.1% to 2.6%) (Fig. E3D [total IBR free rate curves]).

Elevated Risk group (DS > 2.8 without RRt), IBR rates, and RT benefit

Among evaluable patients treated without RT, those in the Elevated Risk group had a clinically elevated IBR rate; the 10-year total IBR rate was 20.6% (95% CI, 13.7%-30.3%) (Fig. 1A), and the 10-year invasive IBR rate was 10.9% (95% CI, 5.8%-19.9%). After BCS, patients treated with adjuvant RT had lower IBR rates than did patients treated without RT (total $P_{\text{logrank}} < .001$; invasive $P_{\text{logrank}} = .003$) (Table E4), where the absolute reduction in the 10-year total IBR rate was $\Delta = 15.7\%$ (95% CI, 7.0%-24.3%) with RT versus without RT (Fig. E3B). Patients treated with BCS plus RT had a lower total IBR rate relative to those treated without RT in the 0-to-10-year time interval (HR, 0.23; 95% CI, 0.11-0.47) (Table 2), and similar results were found for invasive IBR (HR, 0.28; 95% CI, 0.11-0.69) (Table E4—all follow up). Patients who were treated with RT in the Elevated- and Residual Risk groups had a greater relative reduction in IBR rate than did patients in the Low Risk group (multiplicative interaction of RT by risk group: $P = .05$).

There was not a statistically significant difference in the IBR rates for patients in the Elevated Risk group between those from the RMH/KPNW or UUH/UMASS cohorts (total $P_{\text{logrank}} = .68$; invasive $P_{\text{\_logrank}} = .85$) (Table E6). Among patients treated with BCS without RT from the RMH/KPNW subset, those in the Elevated Risk group had a higher IBR rate compared with those in the Low Risk group (total $P_{\text{logrank}} < .001$; invasive $P_{\text{logrank}} < .001$) (Table E7), where the total IBR rate at 10 years was 20.2% (95% CI, 10.5%-36.7%) for patients in the Elevated Risk group (Fig. 1B). The invasive IBR rate in the Elevated Risk group treated with BCS without RT was 9.7% (95% CI, 3.6%-24.9%) at 10 years (Table E7). Patients treated with BCS without RT in the Elevated Risk group also had a higher relative IBR rate compared with the Low Risk group (Table E7).

Patients in the Elevated Risk group from RMH/KPNW study cohorts treated with adjuvant RT had a lower IBR rate compared with those not treated with RT (total $P_{\text{logrank}} < .001$; invasive $P_{\text{logrank}} = .003$) (Table E8). The 10-year total IBR rate after BCS and RT was 5.9% (95% CI, 3.2%-10.8%), which corresponded to a 14.3% (95% CI, 1.0%-27.5%) lower 10-year total IBR rate for patients treated with RT (Fig. 1B and Fig. E3E for IBR free rate curves). The 10-year invasive IBR rate after BCS and RT was 3.8% (95% CI, 1.7%-8.3%), which corresponded to a 7.6% (95% CI, −4.3% to 19.5%) lower 10-year invasive IBR rate for patients treated with RT (Table E8). Patients treated with BCS plus RT had a lower relative total IBR rate (total HR, 0.28; 95% CI, 0.11-0.69) and tended to be lower for invasive IBR compared with those treated without RT in the 0-to-10-year period (invasive HR, 0.34; 95% CI, 0.10-1.22) (Table 2 and Table E8 for all follow-up).

Association of clinicopathologic factors and biosignature risk groups with IBR rate

A multivariable Cox regression model that included key clinicopathologic factors (age, grade, size), biosignature risk groups, and treatment showed that none of the clinicopathologic factors were significantly uniquely associated with total IBR ($P > .12$) in all evaluable patients (Fig. 2A) and in the RMH/KPNW cohort subset (Fig. 2B and Table E9). The biosignature risk groups, RT, and ET treatment continued to be uniquely associated with the total IBR rate for all evaluable patients and the RMH/KPNW subset analysis after including clinicopathologic factors. Multivariable analysis results with a reduced number of covariates, assessing 1 clinicopathologic factor at a time or ET along with biosignature risk groups and RT, were also consistent with the multivariable analysis results including all covariates (data not presented).

Of the common clinicopathologic factors and treatments, nuclear grade and radiation therapy had a statistically significant association with IBR rate as assessed by multivariable Cox regression analysis that excluded the biosignature risk groups (data not presented). The comparison of a first model for total IBR rate that included only these clinicopathologic factors and treatment terms with a second model for total IBR rate that included the biosignature risk groups, treatment and risk group interaction, and clinicopathologic factor terms by likelihood ratio showed that the biosignature risk groups added new information ($P = .012$) and were not simply a surrogate for adverse clinicopathologic features.

As previously noted, patients treated with BCS without RT who were classified into the Elevated- or Residual Risk categories had higher IBR compared with the Low Risk group rates. Similarly, increasing DS on a continuous basis was associated with an increasing IBR rate for patients treated with BCS without RT (total HR per 5 DS units: 3.4; 95% CI, 2.1-5.5; invasive HR per 5 DS units: 3.9; 95% CI, 1.7-9.1). However, for patients treated with BCS plus RT,

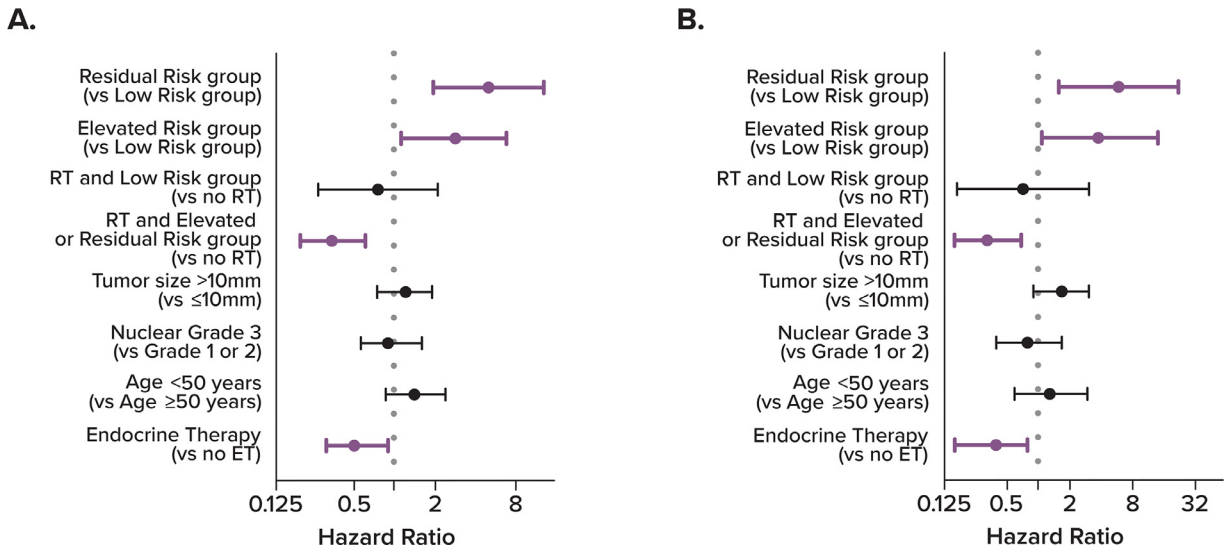

Fig. 2. Forest plot of relative total in-breast recurrence (IBR) rates for biosignature risk groups, clinicopathologic factors, and treatments. Cox proportional hazards multivariable analysis of total IBR rates with biosignature risk groups (Residual Risk and Elevated Risk vs Low Risk groups), clinicopathologic factors, radiation therapy (RT) within risk groups (RT vs no RT in the Low Risk group and in the Elevated- or Residual Risk group), and endocrine treatment. Biosignature risk groups: Low Risk group (decision score [DS] < 2.8 without residual risk subtype [RRt]), Elevated Risk group (DS > 2.8 without RRt), and Residual Risk group (DS > 2.8 with RRt). (A) All evaluable patients (whole cohort). (B) RMH/KPNW cohort subset. *Abbreviations:* CI = confidence interval; HR = hazard ratio.

increasing DS was not associated with a statistically significant relative difference in IBR rate (total $P_{wald} = .68$; invasive $P_{wald} = .68$) when excluding those in the Residual Risk group.

Discussion

As outcomes for early-stage breast cancer (including DCIS) have improved during the past decade, clinicians have been challenged to identify those patients for whom treatment de-escalation may be appropriate. Concurrently, research has focused on moving beyond traditional clinical and pathologic factors to identify those patients who require treatment intensification and/or modification to improve suboptimal outcomes. The current study in women diagnosed with DCIS was conducted to help identify these 3 populations using the novel biosignature combined with DCISionRTas follows: (1) a low-risk (DS ≤ 2.8 without RRt) population of patients who have a low recurrence risk after BCS and can safely omit RT; (2) an Elevatedisk (DS > 2.8 without RRt) population of patients who have an elevated risk of recurrence after BCS that is substantially reduced with adjuvant RT, yielding a low 10-year recurrence risk; and (3) a residual-risk (DS > 2.8 with RRt) population of patients who have an elevated recurrence risk after treatment with standard BCS plus RT. These findings are consistent with previous studies evaluatingDCISionRT, demonstrating that patients diagnosed with DCIS do not have uniform risk after BCS nor uniform benefit from adjuvant RT. The major new finding of the current study is the identification of a third, unique population of patients with a less than optimal response to RT. Together, these findings may be useful, helping clinicians individually tailor treatment; for example, low-risk patients may be counseled to omit adjuvant RT, elevated-risk patients may be counseled to receive standard adjuvant RT, and residual-risk patients may be considered for tumor bed boost, additional systemic therapies, and/or clinical trials.

Previously published studies have suggested that RT benefits patients with DCIS after BCS, with a 50% relative risk reduction in local recurrence with no survival advantage. Additional studies have also attempted to identify patients with low-risk DCIS based on clinical, pathologic, and treatment-related factors. The Eastern Cooperative Oncology Group (ECOG) 5194 study in patients with DCIS included 2 low-risk cohorts (grade 1-2 and grade 3); however, at 12 years, the grade 1-2 cohort had a 14% local recurrence rate, whereas the grade 3 cohort approached 25% with the omission of RT despite wide margins (3 mm or greater).[4] Similarly, in the Radiation Therapy Oncology Group (RTOG) 9804 trial, similar groups of patients were randomized to receive RT or not after BCS; the study found that omission of RT was associated with increased rates of local recurrence (15.1% vs 7.1% at 15 years).[5] Together these studies demonstrate that traditional clinical and pathologic features are insufficient to consistently identify patients with low-risk DCIS for whom omission of RT may be appropriate given the effects on quality of life with local recurrence and the potential effect on breast cancer mortality with

invasive recurrences.[2] In contrast, the present analysis does identify such a Low Risk group with no to minimal benefit from RT. Moreover, in the Low Risk group, the 10-year IBR risk and contralateral breast event rates were quite similar, demonstrating that recurrence rates return to baseline (new primary) risk. Our findings were further confirmed by comparing our multivariable analysis for the clinicopathologic features alone to the clinicopathologic features with the DCISionRT with RRt biosignature risk groups. The results of our analysis indicated that addition of the biosignature risk groups in the multivariate analysis added significant additional information. In addition, a sizeable percentage of patients with nuclear grade 3 were classified as either low risk or elevated risk (without residual risk), thus indicating that DCIS nuclear grade 3 was not adequate alone to discriminate patients to be classified in the Residual Risk group. Collectively, these results suggest that clinicopathology alone has limited capacity to identify patients who have higher recurrence risk after BCS plus RT.

Despite excellent outcomes with respect to local recurrence and survival in modern series, a common concern among clinicians is identifying patients with DCIS who have poor long-term outcomes, including higher rates of local recurrence and the potential for breast cancer mortality.[13,20] Identifying high-risk patients who do not benefit significantly from adjuvant RT can allow for intensification or alteration of therapy to potentially mitigate risk.[2] As such, our results present a key new finding of residual-risk subtypes in patients with DCIS, wherein patients with higher decision scores without a residual-risk subtype (Elevated Risk group) would be expected to substantially benefit from adjuvant RT with much better outcomes than those in the Residual Risk group. The results in this analysis support our hypothesis, demonstrating that in this population of patients treated at 4 different breast cancer centers, DCISionRT with integrated RRt is predictive of radiation therapy response and identifies patients with a suboptimal outcome after BCS with RT.

Recently, the B-43 phase 3 trial by the National Surgical Adjuvant Breast and Bowel Project (NSABP) attempted to evaluate whether HER2 positivity could identify patients with high-risk DCIS and if the addition of trastuzumab (in addition to adjuvant RT) would mitigate this risk. The study was powered to detect a 36% reduction in ipsilateral breast tumor recurrence; however, owing to a limited number of events, the trial demonstrated only a nonsignificant but potentially clinically relevant reduction of 19%.[21] Given this 19% reduction in ipsilateral breast tumor recurrence, the results of B-43 suggested that HER2 may be associated with identifying this cohort of patients with high-risk DCIS. Based on the present study results, one may expect that some but not all patients with HER2-mediated DCIS may be at higher-than-expected risks of recurrence after BCS plus RT, because they were overrepresented in the Residual Risk group. Although patients with an elevated 10-year risk of recurrence after BCS (DS > 2.8) may benefit from adjuvant RT, only the subset of patients classified as residual-risk (DS > 2.8 with RRt) after standard BCS plus RT may be expected to benefit substantially from intensified therapy, such as the HER2 directed therapy evaluated in the NSABP-43 trial. Importantly, 44% of patients with HER2(3+) expression were classified into the Low Risk or Elevated Risk group. Based on the present study results, for patients with HER2(3+) disease, only the 56% in the Residual Risk group may require intensified or alternative therapy, compared with HER2(3+) patients without RRt. These findings provide context for the aforementioned NSABP B-43 outcomes; the nonsignificant 19% benefit seen in B-43 may be owed to inclusion of HER2(3+) patients without RRt (Low Risk or Elevated Risk groups) who may receive less benefit from the addition of trastuzumab compared with patients in the Residual Risk group. Moving forward, potential strategies may be considered to intensify therapy in these patients in the Residual Risk group (DS > 2.8 with RRt), including surgical considerations (wider margins, mastectomy), increased use of boost or modified boost dose, and HER2 targeted therapies (for HER2[3+] patients).

There are limitations to the present analysis. Two cohorts (UUH/UMASS) used in the study were part of the initial development and cross-validation of the DS biosignature, and 2 cohorts (RMH/KPNW) were used for independent validation of DS biosignature. However, none of these cohorts were previously evaluated for residual-risk subtype, and the IBR rates for patients from the UUH/UMASS cohorts and the RMH/KPNW cohorts by biosignature risk group and RT treatment were not statistically different. Additionally, the analysis was not propensity adjusted, introducing the potential for selection bias, whereas the number of events compared with the number of variables in the multivariable analysis may lead to overfitting.

Patients were treated during a 15-year period, and treatment techniques and published outcomes evolved during that time. However, this observation period was needed to provide long-term outcomes, consistent with previous studies reporting long-term outcomes in patients with DCIS. Additionally, treatment was not randomized or driven by DCISionRT testing. However, this study is clinically meaningful because it included patients with DCIS commonly seen in clinics and stratified them based on DCISionRT biologic signatures without affecting the therapies they received, providing outcomes with and without RT for low-, elevated-, and residual-risk populations. Finally, hormone therapy was not consistently used in the present study; however, this is consistent with previous studies that looked at omission of RT, which had rates of hormone therapy of 30% to 60%.[4-6]

Conclusions

DCISionRT testing integrated with a novel residual-risk subtype identified a group of patients with elevated recurrence risk remaining after BCS and RT, independent of traditional clinical and pathologic features, warranting

potential intensified or alternate therapy. In contrast, patients in the Elevated Risk group (without the residual-risk subtype) benefited substantially from RT and had excellent outcomes after RT. The test also identified a group of low-risk patients with excellent outcomes with BCS alone, with no to minimal RT benefit.

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