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Limitations in the Application of Clinicopathologic Factors Alone in Predicting Radiation Benefit for Women With Low-Risk Ductal Carcinoma In Situ After Breast Conserving Surgery: The Impact of a 7-Gene Biosignature Based on 10-Year Ipsilateral Breast Recurrence Rates

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Purpose: Clinicopathologic (CP) factors are used to estimate 10-year ipsilateral breast recurrence (IBR) risk and inform shared decision making regarding postoperative radiation therapy (RT) for ductal carcinoma in situ (DCIS) patients. This study assesses the clinical value of the 7-gene biosignature (DCISionRT) compared to traditional CP definitions for predicting IBR rates and RT benefit.

Methods and Materials: DCIS patients (n = 926) treated with breast conserving surgery (BCS) ± RT were categorized as CP low-risk or high-risk based on established CP factors, study criteria, and nomograms. Women were classified as molecular Low Risk or High Risk by the biosignature. Ten-year IBR rates for CP risk groups were compared with and stratified by the biosignature.

Results: There were 37% of women classified as molecular Low Risk by the biosignature, an average of 47% were classified as low-risk by various CP definitions (range, 35% to 60%). Overall, CP low-risk groups had a mean absolute IBR benefit with RT of 6% (hazard ratio, 0.46; $P < .001$). The biosignature reclassified 53% of CP low-risk patients to molecular High rRisk. These reclassified patients experienced higher IBR rates when RT was omitted and benefited from RT (hazard ratio, 0.30; $P < .001$) with an absolute reduction of 11.6% (17.7% vs 6.1%). CP low-risk patients with concordant biosignature Low Risk demonstrated no significant RT benefit. On average, 28% of high-risk CP patients were reclassified as biosignature Low Risk and had no significant RT benefit (5.9% vs 4.0%).

Conclusions: This observational study supports optimizing de-escalation/escalation treatment strategies for DCIS, the 7-gene biosignature reliably discriminating a LowRisk group without significant RT benefit compared with CP factors alone and a High Risk group that benefited from RT, facilitating improved shared decision making. A randomized clinical trial (NRG CC-016) will provide level 1A evidence for the impact of RT treatment on IBR rates for patients in the 7-gene biosignature Low Risk group, including those with CP high risk.

Introduction

Ductal carcinoma in situ (DCIS) represents a significant percentage of new breast cancer diagnoses in the United States, ~60,000 new cases per year.[1] DCIS lesions can range from slow growing to nearly invasive tumors. Most patients with DCIS choose breast conserving therapy or mastectomy. Overall, DCIS outcomes are excellent with breast conserving therapy, with current 10-year ipsilateral breast recurrence (IBR) rates less than 10%,[1] and very low long-term breast cancer mortality rates of 3%.[2] However, these excellent outcomes create concerns for overtreatment and suggest a need for safe deintensification approaches.

Postoperative radiation therapy (RT) is a mainstay in the management of DCIS for patients undergoing breast conserving surgery (BCS), with studies consistently demonstrating a 50% relative reduction in IBR rates independent of clinicopathologic (CP) factors, with no survival advantage noted.[3] Given the lack of survival benefit, studies have evaluated omission of RT following BCS to de-intensify therapy. The Eastern Cooperative Oncology Group (ECOG) 5194 trial evaluated the omission of RT following BCS for grades 1-2 and 3 cohorts, respectively. Although initial outcomes were promising, long-term follow-up demonstrated substantially elevated rates of IBR with approximately 15% of grade 1-2 and 25% of grade 3 patients developing an IBR at 12 years, and no plateau in recurrences was noted.[4] Similar results were seen in other studies, including the Radiation Therapy Oncology Group (RTOG) 9804 trial, which randomized patients with favorable CP features to receive RT or not following BCS and found elevated rates of recurrence at 15 years with the omission of RT (15% vs 7%).[5,6] However, currently, RTOG 9804 criteria are recommended in the National Comprehensive Cancer Network (NCCN) guidelines to identify low-risk.[7]

Taken together, the results of these studies demonstrate that CP criteria alone are unable to reliably identify a low-risk cohort of patients with DCIS that do not benefit from RT following BCS. Beyond CP criteria, nomograms have been developed, including the Memorial Sloan Kettering Cancer Center (MSKCC) nomogram for DCIS and the Van Nuys Prognostic Index (VNPI). While studies demonstrate these nomograms may be prognostic with respect to IBR, there is a lack of consistent external validation evidience, and they are not predictive of RT benefit.[8-13]

Previous studies have identified molecular biomarkers that are prognostic for IBR, but not predictive for RT benefit. A 12-gene assay, the Oncotype DX DCIS Score provides an estimated risk of 10-year total and invasive IBR but does not predict the benefit of RT.[14] In a Canadian population-based data set, the relative IBR reduction with RT in patients treated in the most recent time period was 50%, in both low-risk and high-risk Oncotype groups, which is similar to the relative RT benefit seen in trials using CP criteria.[4,5] In contrast, the 7-gene biosignature (DCISionRT) combines the protein expression for 7 genes with 4 CP factors to provide 10-year IBR risk and predicts RT benefit.[15-19] It has been clinically validated in multiple studies, including the randomized SweDCIS trial, confirming it is prognostic for IBR risk (invasive and noninvasive), while also predicting RT benefit. Studies have demonstrated that biosignature Low Risk patients did not significantly benefit from RT, whereas biosignature High Risk patients did benefit.[15-19] The biosignature has been analytically validated and demonstrated to have significant clinical utility in a large, prospective, multicenter study.[16,20,21]

Despite the failure of CP factors/nomograms to identify a low-risk CP group that does not significantly benefit from RT, clinicians commonly use CP factors alone to inform shared decision-making regarding postoperative RT, despite their failure to predict RT benefit in addition to multiple known confounding factors.[22,23] Inconsistency in the assessment of risk and treatment benefit based on CP factors generates significant regional/institutional heterogeneity in treatment.[24,25] Further complicating decision making, RT treatment can vary with intent to treat with endocrine therapy (ET) and women receiving ET may be 30% less likely to receive postoperative RT.[26]

Previously, it was reported that the 7-gene biosignature provided additional information beyond CP factors in multivariable analysis (MVA) and that the biosignature reclassified patients based on clinicopathology factors and criteria, but a full analysis of the interactions between clinicopathology criteria and the biosignature was beyond the scope of the prior publications.[17]

In this new analysis of the same cohorts, the 10-year IBR rates with and without RT for patients with low-risk and high-risk CP based on combinations of individual CP factors, CP criteria used in key trials (ECOG 5194 and RTOG 9804), and the Van Nuys Prognostic Index (VNPI) and Memorial Sloan Kettering Cancer Center (MSKCC) nomograms were evaluated to illustrate the challenges of using these techniques as part of shared decision making to adequately inform patients with DCIS. To understand the impact of the biosignature as compared with CP-based risk assessment, the rates of IBR and the impact of RT when these CP cohorts were re-stratified by the 7-gene biosignature risk groups as concordant or discordant were assessed.

Methods and Materials

The 7-gene biosignature was previously validated to be both prognostic of the IBR rate after BCS without RT and predictive of the benefit from BCS plus or minus RT.[15,18,19] Recently, a novel molecular residual risk subtype (RRt) was also validated to identify DCIS patients with a high-risk of IBR after BCS and RT in a multisite cohort.[17] However, these prior validation studies did not include a detailed comparison between the biosignature and common clinicopathology-based risk assessments. Here, we directly compare risk assessment based on common clinicopathologic criteria and the 7-gene biosignature in the same evaluable patient population that was previously reported,[17] which is comprised of the 2 cross-validation cohorts from Uppsala University Hospital and Västmanland County Hospital Sweden (UUH) and University of Massachusetts Worcester (UMASS) and 2 independent external validation cohorts, Kaiser Permanente Northwest (KPNW) and The Royal Melbourne Hospital and Royal Women's Hospital, Parkville, Australia (RMH) and separately in the subset of independent external validation cohorts.

This multi-institutional study consisted of 926 patients as previously reported; this present study represents new analyses from this data set beyond the scope of prior publications.[17] IBR was defined as either a subsequent ipsilateral DCIS or an invasive recurrence, excluding metastatic events. We used the first qualifying IBR at least 6 months after the primary DCIS diagnosis as the study outcome. The 7-gene biosignature (PreludeDx, Laguna Hills, CA) integrates the protein expression for 7 genes (COX-2, FOXA1, HER2, Ki-67, p16/INK4A, PR, and SIAH2) and 4 CP factors (age at diagnosis, tumor size, palpability, and surgical margin status), using a nonlinear algorithm[15] (Fig. E1).

The Residual Risk subtype (RRt) is a second nonlinear algorithm within the 7-gene biosignature. The RRt combines a subset of the same molecular biomarkers but does not include CP factors. The RRt identifies patients with HER2/KRAS pathway-driven tumors who have a high IBR rate after BCS and an elevated IBR rate after BCS plus RT. The test reports a continuous risk score (decision score [DS]) and the RRt, validated to be associated with the risk of local recurrence after BCS and the expected reduction in the recurrence risk with postoperative RT.[15-19]

Biosignature testing was conducted on archived tissue samples as previously reported.[17] In this analysis, women were classified as DS Low Risk (DS $\leq$ 2.8, no RRt) or DS High Risk (DS $>$ 2.8, with or without RRt). Validation of the 7-gene biosignature in the SweDCIS randomized clinical

Table 1 Definition of CP low-risk and high-risk groups

CP-based risk assessment	CP high-risk group definition	CP low-risk group definition	Score definition
RTOG 9804-like[1]	Grade 3, or size >2.5 cm, or palpable, or clinically detected	Grade 1 or 2, and size ≤2.5 cm, and nonpalpable, and screen detected, and negative margin*	NA
ECOG 5194-like	Grade 3 or size >2.5 cm	Grade 1 or 2, and size ≤2.5 cm, and negative margin*	NA
VNPI-like[2,3]	Score ≥7	Score <7	Weighted size, nuclear grade, necrosis, age, and margins (VNPI 2003); however, negative margin status defined as no ink-on-tumor.
MSKCC DCIS nomogram-like[4]	Score >220	Score ≤220	Weighted hormone therapy, nuclear grade, necrosis, screening detection, necrosis, age, family history, and margins; however, number of excisions not used, and negative margin status was defined as no ink-on-tumor. RT was not included as a factor.
Age and grade	Age <50 or grade 3	Grade (1 or 2) and age >50	NA

Abbreviations: CP = clinicopathologic; DCIS = ductal carcinoma in situ; RTOG = Radiation Therapy Oncology Group; ECOG = Eastern Cooperative Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; RT = radiation therapy; VNPI = Van Nuys Prognostic Index; NA = not applicable.
* negative margine defined as 'no ink on tumor'.

trial cohort previously defined the optimal threshold of DS=2.8 to stratify a DS low risk (DS ≤ 2.8) group, who had minimal to no benefit from RT, from the remaining patient group (DS > 2.8), who had a significant reduction from RT.[17,18]

CP factors were combined using age, grade, size, and margin status as well as criteria based on VNPI, MSKCC DCIS nomogram, ECOG 5194, or RTOG 9804 studies (Table 1).[1-4] For these analyses, the predefined RTOG 9804 and ECOG 5194 criteria for low-risk DCIS were used, except for margin status, which defined a negative margin as "no ink on tumor" for the present study.

Published criteria were used to define MSKCC-like low-risk patients (score ≤ 220 corresponding to a predicted 10-year IBR rate of 10%),[9] with the caveat that margin status was only negative vs. positive (based on 'ink on tumor') and that our calculation did not include 3 or more repeat surgeries as a risk factor (Table 1). Prior studies have indicated that patients requiring 3 or more excisions are expected to have low incidence.[27] Some subjects in our cohort had missing data on family history. Similarly, published criteria were used to define VNPI-like low-risk (score < 7) but margin status was only assessed as 'no ink on tumor'[28] (Table 1). Use of postoperative endocrine therapy was also recorded if prescribed.

Risk analyses

Patients meeting low-risk CP criteria were classified as "low-risk"; otherwise, they were classified as "high-risk" for each CP group (Table 1). The different CP low-risk and high-risk groups were further stratified into dichotomous DS Low Risk and DS HighRisk groups by the 7-gene biosignature. Biosignature risk group distributions were determined within patients meeting the various low-risk and high-risk CP criteria. Kaplan−Meier survival analysis was used to calculate the IBR rates for patients treated with and without postoperative RT and assessed using the log-rank test to determine whether the curves were distinct. Cox proportional hazards analysis was used to calculate the hazard ratios (HRs) corresponding to postoperative RT.

Univariable and multivariable Cox proportional hazards analyses were also used to assess whether CP-based risk assessments were associated with IBR rates after accounting for biosignature results and treatment, including continuous nomogram scores. Hazard ratios for CP risk assessment were assessed by meta-analysis of the various risk groups (Table 1) and a pooled analysis of individuals belonging to any of the risk groups was used to calculate pooled survival statistics. 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 and institutional review boards for for Uppsala University Hospital and Västmanland County Hospital, Sweden (UUH), University of Massachusetts Worcester (UMASS)[15] and 2 independent validation sites, Kaiser Permanente Northwest (KPNW) and The Royal Melbourne Hospital and Royal Women's Hospital, Parkville, Victoria Australia (RMH).[15,17,29] Biostatistical analyses were independently completed by McCloud Consulting Group, Australia.

Table 2 10-year IBR rates and RT difference for low-risk CP patient groups compared with the DS low-risk patient group for the evaluable cohort

		10-y IBR rates		Absolute RT difference		Relative RT difference	
CP "low-risk" criteria	n (%)	no RT (95% CI)	RT (95% CI)	Abs diff (95% CI)	*P*-log rank	HR 95%CI	*P* Wald
RTOG 9804-like low-risk	473 (51%)	12.1% (7.2%, 19.9%)	6.2% (3.8%, 10.1%)	5.9% (1.0%, 12.8%)	.034	0.47 (0.23, 0.96)	.039
MSKCC-like <220	320 (35%)	8.3% (4.0%, 16.8%)	4.7% (2.3%, 9.4%)	3.6% (3.3%, 10.4%)	.12	0.45 (0.16, 1.26)	.13
VNPI-like <7	359 (39%)	12.4% (7.4%, 20.6%)	4.5% (2.2%, 8.8%)	7.9% (0.9%, 15.1%)	.013	0.36 (0.15, 0.84)	.018
Age >50 and Grade 1 or 2	453 (49%)	13.3% (8.2%, 21.3%)	6.8% (4.2%, 11.1%)	6.5% (0.7%, 13.7%)	.034	0.48 (0.24, 0.94)	.034
ECOG 5194-like grade 1 or 2	553 (60%)	13.1% (8.4%, 20.0%)	6.6% (4.3%, 10.2%)	6.5% (0.1%, 12.8%)	.022	0.49 (0.26, 0.91)	.025
Mean [min, max]	47% [35%, 60%]	11.8% [8.3%, 13.3%]	5.8% [4.7%, 6.8%]	6.0% [3.6%, 7.9%]	NA	0.46* (0.33, 0.64)	<.001*
Pooled analysis Any low-risk CP†	682 (74%)	13.8% (9.3%, 20.4%)	5.9% (3.9%, 9.0%)	7.9% (2.0%, 13.9%)	.001	0.39 (0.2, 0.7)	.002
DCISionRT Low risk	**338 (37%)**	**5.6% (2.0%, 12.0%)**	**4.8% (2.5%, 9.1%)**	**0.8% (4.6%, 6.2%)**	**.71**	**0.80 (0.5, 1.5)**	**.71**

Abbreviations: CP = clinicopathologic; DCISionRT = Ductal Carcinoma in Situ (DCIS) decision radiation therapy; HR = hazard ratio; RTOG = Radiation Therapy Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; ECOG = Eastern Cooperative Oncology Group; RT = radiation therapy; VNPI = Van Nuys Prognostic Index; NA = not applicable.

Bold values are the summary statistics of the 7-gene biosignature DCISionRT.

* Mean HR (95% CI) and *P* value were calculated using meta-analysis.

† Analysis of the pooled subset of subjects that satisfy any of the 5 CP high-risk criteria.

Results

The performance of the 7-gene biosignature was compared with common clinicopathologic (CP) risk criteria in the evaluable study population, which comprised patients diagnosed with DCIS from 4 cohorts (2 cross-validation cohorts UUH, UMASS and 2 independent external validation cohorts KPNW, RMH). Overall, 69% of the evaluable study population received postoperative RT and 34% were prescribed postoperative ET; 73% of patients who were prescribed ET also received RT. Mean follow-up for the evaluable study population was 8.8 years (median, 8.5 years; first to third quartile, 5.8-10.2 years), with a total of 92 ipsilateral breast events recorded overall.

Overall, about half of patients (mean 47%) in the evaluable population were classified as low-risk by the various CP-based criteria listed in Table 1 (range, 35%-60%; Table E1). The distribution of these low-risk and high-risk CP profiles did not differ significantly between the 2 cross-validation UUH/UMASS and the 2 independent external validation cohorts KPNW/RMH (Table E1). The individual CP factors and treatment characteristics were also summarized by CP risk profiles and 7-gene biosignature risk groups (Tables E2 and E3).

The distribution of the individual CP factors varied between the CP risk groups defined by different criteria. Generally, younger patients or those with high grade or larger tumors were often classified as having high-risk CP. However, in some instances, criteria like the VNPI or the MSKCC nomogram classified these same patients as having low-risk CP.

Comparison of risk assessments by clinicopathology and 7-gene biosignature

Both patients with low-risk and high-risk CP profiles benefitted significantly from adjuvant RT when assessed independently of 7-gene biosignature test results (Tables 2 and 3). Patients with high-risk CP profiles had approximately 2 times greater IBR rates than those with low-risk CP profiles after treatment with BCS without RT (mean 10-year IBR rates 11.8% vs 22.5%). As expected, those patients with high-risk CP profiles benefited from RT (HR 0.28; $P < .001$) with corresponding mean 10-year IBR of 22.5% after BCS without RT and 7.7% with RT (Table 3). However, patients with low-risk CP profiles also benefited from RT (HR 0.46; $P < .001$) with a corresponding mean 10-year IBR of 11.8% after BCS without RT and 5.8% after BCS with RT (Table 2). Similar results were observed for the KPNW/

Table 3 10-year IBR rates and RT benefit for different high-risk CP patient groups compared with the DCISionRT high-risk patient group for the evaluable cohort

		10-y IBR rates		Absolute RT difference		Relative RT difference	
CP "high-risk" criteria	N (%)	no RT (95% CI)	RT (95% CI)	Abs diff (95% CI)	*P*-log rank	HR (95%CI)	*P*Wald
RTOG 9804-like high-risk	453 (49%)	23.6% (16.4%, 33.2%)	7.60% (4.9%, 11.8%)	16.0% (7.0%, 24.9%)	<.001	0.27 (0.15, 0.49)	<.001
MSKCC-like >220	606 (65%)	21.1% (15.4%, 28.6%)	8.0% (5.5%, 11.6%)	13.1% (5.9%, 20.3%)	<.001	0.34 (0.20, 0.55)	<.001
VNPI-like ≥7	567 (61%)	22.6% (15.8%, 31.9%)	8.1% (5.6%, 11.8%)	14.5% (6.0%, 23.0%)	<.001	0.30 (0.17, 0.51)	<.001
Age <50 or Grade 3	473 (51%)	22.6% (15.5%, 32.2%)	6.9% (4.5%, 10.7%)	15.7% (6.8%, 24.5%)	<.001	0.26 (0.14, 0.47)	<.001
Not ECOG 5194-like (grade 3 or size >2.5 and grade 1 or 2)	373 (40%)	25.9% (17.4%, 37.6%)	7.4% (4.5%, 12.0%)	18.5% (7.9%, 29.2%)	<.001	0.22 (0.11, 0.41)	<.001
Mean [min, max]	494 (53%)	22.5% [21.1%, 25.9%]	7.7% [6.9%,8.1%]	15.6% [13.1%, 18.5%]	NA	0.28* (0.22, 0.37)	<.001*
Pooled analysis Any high-risk CP†	817 (88%)	18.0% (13.2%, 24.4%)	7.3% (5.2%, 10.2%)	10.7% (4.7%, 16.8%)	<.001	0.36 (0.22, 0.57)	<.001
DCISionRT High risk	**588 (63%)**	**25.7% (19.0%, 34.0%)**	**8.0% (6.0%, 12.0%)**	**17.7% (9.4%, 26.0%)**	**<.001**	**0.25 (0.15, 0.42)**	**<.001**

Abbreviations: CP = clinicopathologic; DCISionRT = Ductal Carcinoma in Situ (DCIS) decision radiation therapy; HR = hazard ratio; RTOG = Radiation Therapy Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; ECOG = Eastern Cooperative Oncology Group; RT = radiation therapy; VNPI = Van Nuys Prognostic Index; NA = not applicable.

Bold values are the summary statistics of the 7-gene biosignature DCISionRT.

* Mean HR (95% CI) and *P* values were calculated using meta-analysis.

† Analysis of pooled subset of subjects that satisfy any of the five CP high-risk criteria.

RMH independent external validation cohorts (Tables E4 and E5).

Patients with discordant risk profiles

Overall, the majority (mean: 56%) of patients for each of the low-risk CP profiles were reclassified as DS High Risk (range: 51% to 63%) in the evaluable population (Fig. 1A; Table E6). For each of the low-risk CP criteria, those patients reclassified as DS High Risk had significantly reduced IBR rates with RT (vs without RT) with HRs varying from 0.23 ($P = .005$) to 0.35 ($P = .012$), where the mean HR was 0.30 ($P < .001$). For example, 51% of patients who met RTOG 9804-like criteria were reclassified as DS High Risk and benefited from RT (HR 0.30; $P = .007$), with corresponding 10-year IBR rates of 19.5% without RT and 6.8% with RT. Overall, patients who met any of these 5 low-risk CP criteria and were reclassified to DS High Risk had a mean 10-year IBR rate of 21.4% without RT and 6.4% with RT. Similar results were observed for the KPNW/RMH independent external validation cohorts subset (Fig. 1B; Table E7), where overall, the majority (mean 54%) of patients with low-risk CP were reclassified as DS High Risk and their IBR rate was significantly reduced by RT with a mean HR of 0.32 ($P < .001$).

Of those patients who had high-risk CP profiles, a mean of 28% were reclassified as DS Low Risk (range: 22% to 33%) in the evaluable population (Fig. 2A; Table E8). For those patients reclassified as DS Low Risk, IBR rates did not differ significantly with RT versus without RT for any of the high-risk CP criteria (*P* values ranged from .26 to .70). For example, 23% of patients who had a high-risk RTOG 9804-like profile were reclassified as DS Low Risk and did not have a significant reduction in IBR from RT ($P = .52$), where the corresponding 10-year IBR rates were 5.9% without RT and 3.0% with RT. Overall, patients who met any of these high-risk criteria and were reclassified to DS Low Risk had a mean 10-year IBR of 5.0% without RT and 5.2% with RT. Similar results were observed for the KPNW/RMH independent external validation cohorts subset (Fig. 2B; Table E9), where overall a mean of 33% of patients with high-risk CP were reclassified as DS Low Risk and their IBR rate was not significantly reduced by RT ($P = .33$).

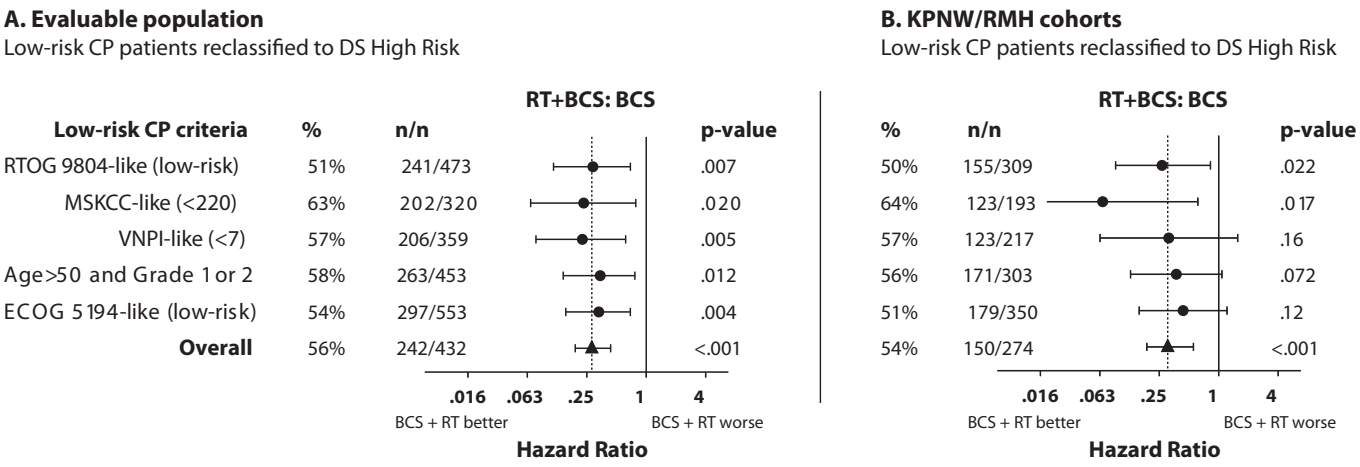

Fig. 1. Forest plot of RT benefit in patients with low-risk CP who were reclassified as 7-gene biosignature High Risk. Low-risk clinicopathology definitions are provided in Table 1. Univariable analyses for each of the 5 low-risk clinicopathology groups are summarized for the evaluable population (A) and for the independent external validation cohorts (B). The percentage of patients with low-risk CP who were reclassified as DS HighRisk and the corresponding numbers of patients in each low-risk clinicopathology group are indicated along with the *P*-value for the HR. The overall results for the 5 low-risk groups reclassified to DS High Risk are summarized by meta-analysis. *Abbreviations:* BCS = breast conserving surgery; CP = clinicopathologic; DS = decision score; HR = hazard ratio; RTOG = Radiation Therapy Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; ECOG = Eastern Cooperative Oncology Group; RT = radiation therapy; VNPI = Van Nuys Prognostic Index.

Patients with concordant risk assessments by CP-based assessment and 7-gene biosignature

On average, 44% of patients (range: 37% to 49%) with low-risk CP profiles had concordant Low-Risk 7-gene biosignature results (Table E10). There was no significant difference in IBR rates by receipt of RT for patients with concordant low-risk CP profiles. Likewise, a mean of 72% of patients (range: 67% to 78%) with high-risk CP profiles had concordant High Risk 7-gene biosignature and significantly benefited from RT (HR, 0.26; $P < .001$; Table E11). Similar

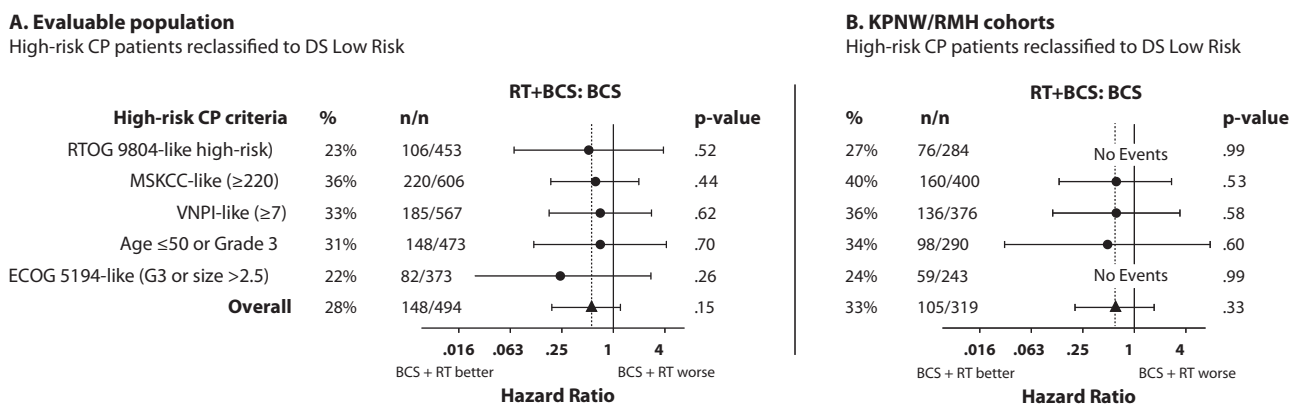

Fig. 2. Forest plot of RT effect in patients with high-risk CP who were reclassified as 7-gene biosignature Low Risk. High-risk clinicopathology definitions are provided in Table 1. Univariable analyses for each of the 5 high-risk clinicopathology groups are summarized for the evaluable population (A) and for the independent external validation cohorts (B). The percentage of patients with high-risk CP who were reclassified as DS Low Risk and the corresponding numbers of patients in each high-risk clinicopathology group are indicated along with the *P*-value for the HR. The overall results for the 5 high-risk groups reclassified to DS Low Risk are summarized by meta-analysis. *Abbreviations:* BCS = breast conserving surgery; CP = clinicopathologic; DS = decision score; HR = hazard ratio; RTOG = Radiation Therapy Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; ECOG = Eastern Cooperative Oncology Group; RT = radiation therapy; VNPI = Van Nuys Prognostic Index.

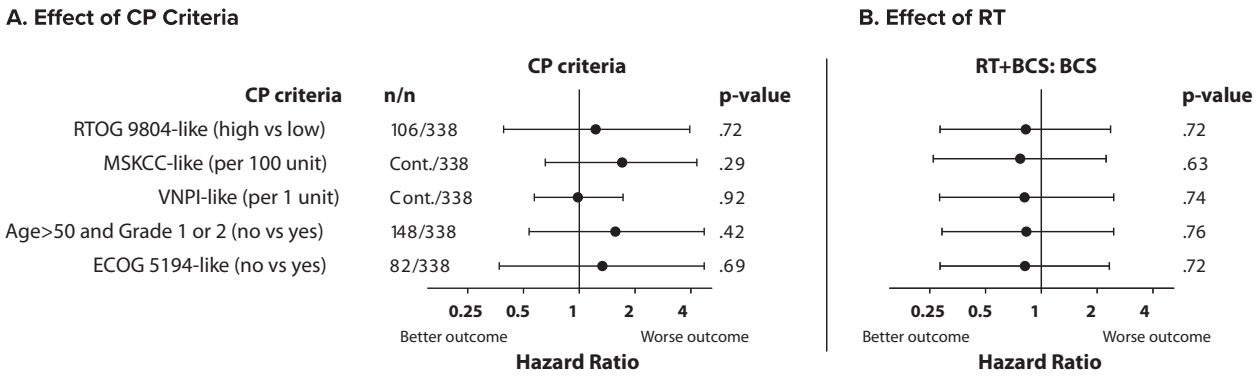

Fig. 3. Forest plot of effect of CP risk criteria on IBR in patients classified as 7-gene biosignature Low Risk. Among patients classified as DS Low Risk, forest plots representing hazard ratios of the effect of CP risk criteria and RT on IBR risk are presented in (A) and (B), respectively. Clinicopathology risk criteria definitions are provided in Table 1. Multivariable analyses for each of the 5 clinicopathology risk criteria are summarized for the patients with a DS Low Risk in the evaluable population. The percentage of patients with high-risk CP in the DS Low Risk group and the corresponding numbers of patients are indicated along with the *P*-value for the HR. The multivariable analysis was performed using the continuous score for MSKCC-like and VNPI-like CP criteria. *Abbreviations:* BCS = breast conserving surgery; CP = clinicopathologic; DS = decision score; HR = hazard ratio; RTOG = Radiation Therapy Oncology Group; MSKCC = Memorial Sloan Kettering Cancer Center; ECOG = Eastern Cooperative Oncology Group; RT = radiation therapy; VNPI = Van Nuys Prognostic Index.

results were observed for the KPNW/RMH independent external validation subset (Tables E12 and E13).

Analyses of individual CP risk factors

When assessing individual low-risk CP factors, either age >70, size ≤1.5 cm, or grade 1 or 2, a mean of 64% (range: 55% to 78%) of patients were re-classified as DS High Risk. Importantly, for each individual low-risk CP factor, the subset of patients reclassified as DS High Risk significantly benefited from RT (HR 0.24; $P < .001$) with corresponding mean 10-year IBR rates of 21% without RT versus 5.4% with RT (Table E14). We also noted that when assessing individual high-risk CP factors, either age <50, or size >2.5 cm, or grade 3, a mean of 47% of patients (range; 22% to 42%) were reclassified to DS Low Risk, and these reclassified patients did not benefit from RT (Table E14).

Multivariable analyses of CP criteria and 7-gene biosignature

When assessed independently of the 7-gene biosignature test results (Table 2), patients with high-risk CP profiles had approximately 2 times greater IBR rates than those with low-risk CP profiles after treatment with BCS without RT. After incorporating 7-gene biosignature test results, none of the CP criteria were significantly associated with IBR rate in multivariable analysis (MVA) (Table E15). However, the continuous 7-gene biosignature DS was associated with IBR. Similar results were observed for the KPNW/RMH independent external validation subset Table E15.

From another perspective, neither CP criteria nor RT were significantly associated with IBR on MVA for patients in DS Low Risk group (Fig. 3). Furthermore, for DS Low-Risk patients, none of the individual CP factors were associated with IBR on MVA Table E16. Thus, patients classified into the 7-gene biosignature Low Risk group did not have a significantly increased or decreased IBR rate because of individual CP factors or CP criteria.

Residual risk subtype and risk remaining after treatment with RT

In the evaluable patient population, the biosignature also identified that 20% of patients had elevated risk remaining after treatment with BCS plus RT. These patients had the Residual Risk subtype (RRt) and were classified into the Residual Risk group. Accounting for the Residual Risk subtype, the biosignature stratified patients with 'low-risk' and 'high-risk' CP profiles into 3 risk groups, with different DS Low Risk, DS Elevated Risk, and Residual Risk group distributions within the CP risk groups (Fig. E2).

Of note, between 5% and 20% of patients with low-risk CP profiles and between 20% and over 40% of patients with high-risk CP profiles had the Residual Risk subtype. Patients with the Residual Risk subtype had a 42% 10-year IBR rate after BCS without RT, which was approximately 8 times greater than the DS Low Risk group after BCS without RT.[17] For those patients treated with RT, the Residual Risk group was also prognostic for IBR risk (HR- 2.5; 95% CI, 1.3 to 5.0; $P = .005$) and had a corresponding elevated IBR rate of 14.7% (95% CI, 8.8% to 24.1%).[17] However, none of the CP criteria were significantly associated with IBR rate after

accounting for the Residual Risk subtype on MVA Table E17. Finally, neither categorical CP criteria nor continuous nomograms based on VNPI, MSKCC DCIS nomogram, ECOG 5194, or RTOG 9804 studies was able to reliably identify the subset of patients with elevated residual risk remaining after BCS plus RT Table E18.

Discussion

This analysis yields several key findings. As expected, a large percentage of patients had discordant risk assessments based on CP criteria or nomograms compared with the 7-gene biosignature results. On average, over half of patients with low-risk CP profiles were reclassified as DS High Risk (51% to 63%) and many patients with high-risk CP profiles were reclassified as DS Low Risk (23% to 32%). Importantly, those patients with discordant CP and 7-gene biosignature risk assessments had clinically meaningful differences in risk reduction with postoperative RT. Overall, patients with low-risk CP profiles benefited from postoperative RT, whereas those with DS Low Risk did not. However, patients with low-risk CP profiles who were reclassified as DS High Risk significantly benefited from postoperative RT (10-year IBR rate 11.6% lower on average with RT), whereas patients with concordant low-risk profiles had no significant benefit from RT. Conversely, those patients who had high-risk CP profiles and were reclassified as DS Low Risk did not benefit from RT. On MVA, after accounting for 7-gene biosignature test results, neither individual CP factors, nor composite CP classifications, nor nomograms evaluated on a continuous basis were significantly associated with IBR. The biosignature differs from CP-based risk assessments in that it incorporates 7 biomarkers and 4 CP factors using a nonlinear algorithm rather than a simple linear combination of factors. The analysis demonstrates that there is a poor concordance between the various CP risk groups and the biosignature risk classification. For example, the low-risk CP groups had high reclassification rates by the biosignature and the re-classified patients had significant RT benefit, indicating that the biosignature provided novel information beyond the assessment based on various CP criteria. Collectively, the results of this analysis affirm that the biosignature can delineate high-risk from low-risk patients with high fidelity independent of CP-based risk assessment. With regards to absolute rates of IBR, it is important to recognize the era in which the patients were treated, given that the rates of local recurrence have consistently declined; as such, a 5.6% IBR rate at 10 years is in line with BCS plus RT from that era.[5] It is also important to note that the 10-year contralateral breast event rate was 4.1% (2.0% to 8.5%) in this study cohort, which is a measure of the expected new primary rate.[17] The randomized trial (NRG CC-016) for patients with DCIS will soon be underway, providing level 1A evidence for modern IBR rates for DS Low Risk patients (DS $\leq$ 2.8) who will be randomized to receive or omit RT.

Just as importantly, our study demonstrates that neither categorical CP criteria nor continuous nomograms based on VNPI, MSKCC DCIS nomogram, ECOG 5194, or RTOG 9804 studies were able to reliably identify the subset of patients with elevated residual risk remaining after BCS plus RT. This is important as these patients represent 20% of cases and may benefit from treatment intensification (eg, tumor bed boost) and more intensive follow-up surveillance; therefore, when relying on CP criteria, clinicians would be unable to successfully identify such patients.

As previously discussed, given the usually excellent outcomes and the potential for toxicities associated with postoperative therapies, the ability to appropriately deintensify treatment for patients diagnosed with DCIS is essential. Today, CP-based criteria like RTOG 9804 are commonly used by clinicians to help identify low-risk patients who may be appropriate for omission of RT, as currently recommended by the NCCN guidelines.[7] However, this analysis suggests that this may lead clinicians astray when used as part of shared decision making, given that more than half of CP low-risk cases are actually biosignature High Risk and have significant benefit from RT. Our study results were similar for other low-risk CP criteria, based on the MSKCC nomogram, VNPI, and ECOG 5194 studies. In contrast, over a quarter of high-risk CP patients were reclassified as biosignature Low Risk, with no benefit to the addition of RT. For example, young age (<50 years) and high nuclear grade are 2 CP factors commonly used to support the recommendation of postoperative RT. While about half of patients are expected to be high-risk by age/grade criteria, this analysis demonstrated that about one third of these patients have DS Low Risk disease, with no benefit to adjuvant RT. Furthermore, for DS Low Risk patients, there was no significant difference in the IBR rate with RT for patients who were young or had high nuclear grade, either independently or jointly. Similar results were obtained for other high-risk CP criteria.

Finally, neither CP criteria nor nomograms were associated with IBR rate after accounting for the continuous biosignature DS result. Taken together, these findings suggest that treatment decisions based on the use of CP features alone may lead to both over- and undertreatment with RT when compared with the use of the biosignature and raises the question of how clinicians can accurately counsel patients regarding the pros/cons of postoperative RT without the biosignature. At this time, population data have demonstrated that approximately two thirds of patients undergoing BCS for DCIS receive RT with significant regional differences.[25,30] The current data, coupled with previous publications utilizing 7-gene biosignature, suggest that 63% of patients with DCIS will be biosignature High Risk, benefiting from the addition of RT. As such, while the proportion of patients receiving RT may not change with the widespread use of the 7-gene biosignature test, what will change is that RT will be given to those most likely to benefit from it, preventing over and undertreatment. Given the widespread use of CP in risk stratification for patients with

DCIS, one must ask why doesn't it work? In addition to the inability of CP factors to adequately identify intrinsic lesion biology,[31] there are several confounding factors identified in previous studies, including differences in pathologist's interpretation of grade,[32-34] tumor size,[34] and surgical margins,[35,36] differences in the application of BCS[36] and RT dose,[37] use and compliance with endocrine therapies,[38] and variabilities in the clinical presentation of cases[38] (ie, clinical vs mammographic presentation).[39]

There are limitations to this analysis. First, this was not a prospective randomized trial but rather a retrospective analysis of a large collection of patients with DCIS with long-term follow-up available from multiple cohorts. Hence, the possibility of selection bias and hidden confounders exists. Notably, the uncontrolled use of RT in these data sets would likely trend toward utilization in CP high-risk patients. Nonetheless, the biosignature retains discriminatory power in both CP low-risk and CP high-risk groups. Additionally, some subsets of patients included smaller numbers of cases and events, and patients in this analysis were treated over different treatment eras (1986-2011). As such, differences in surgical techniques and RT delivery exist introducing heterogeneity among the cohorts. These limitations are addressable with a modern prospective randomized study design.

We also note that the CP criteria used in this study are based on MSKCC, VNPI, RTOG 9804, and ECOG 5194 criteria or continuous nomograms but are not exactly reproducible, as the present study only evaluated margin status as no-ink on tumor and did not include the number of excisions. These adaptations were necessary due to data availability constraints (eg, lack of detailed margin measurements and number of excisions). Previous studies have suggested that margin width may be related to IBR, potentially impacting this analysis. However, a recent analysis of 2,546 patients from the NSABP-B35 trial concluded that there was no clinically meaningful difference among patients with margins <2 mm (n = 879; IBR = 5.3%) versus margins ≥2 mm (n = 1667; IBR = 3.8%).[40] Similarly, BCS requiring 3 or more excisions is a factor in the MSKCC nomogram, but it has a low expected incidence (≤10%).[9,27] Consequently, it is expected that the absence of these 2 factors would not have a material impact on the analysis of average 10-year IBR rates for CP-based risk groups.

Additionally, a detailed analysis of the effect of ET and the interaction with biosignature and CP risk groups is beyond the scope of this analysis and will be addressed separately. Endocrine therapy is expected to result in a 50% reduction in new primary breast cancers.[3,41] In NSABP B24, Tamoxifen was associated with a 3.2% absolute 10-year reduction in contralateral breast cancer after lumpectomy and RT for DCIS.[42] A previous analysis[43] in this group of patients demonstrated a small IBR reduction with ET for patients in the DS Low Risk group treated with or without RT, which was consistent with the reduction in new primary cancers seen in the contralateral breast.[44] However, beyond this apparent reduction in new primary cancer recurrences, no significant reduction was seen in the DS Low Risk patients with or without RT. There was an apparent IBR reduction beyond the expected reduction in new primary cancers in patients in the DS High Risk group treated with ET without RT, but not in DS High Risk patients receiving RT. This finding is in line with those reported from the overview of DCIS randomized trials,[3] where patients receiving RT had a small reduction in IBR with ET but those not receiving RT had approximately half of the reduction in IBR seen with RT, when receiving adjuvant ET alone. Little or no reduction beyond that expected in new primary cancers is likely with ET in the RRt group, since those tumors are driven by an activated HER2/KRAS pathway, independent of ER expression.[17]

To address these limitations, a prospective randomized clinical trial (NRG CC-016) will soon be underway that includes random assignment of RT treatment in biosignature Low Risk patients, to ensure that both known and unknown confounding factors are distributed evenly between treatment groups. This study will provide level 1A evidence to assess the difference in IBR rate between patients in the DS Low Risk group (DS ≤ 2.8) treated with and without RT and allowing the impact of clinicopathology on the IBR rate to be investigated in further detail.

Conclusions

Collectively, the results of this retrospective analysis highlight limitations of CP-based risk assessment alone and indicate that the 7-gene biosignature can delineate high-risk from low-risk patients with high fidelity independent of CP-based risk assessment. The biosignature reclassified more than half of patients who would be classified as low-risk by CP criteria as DS High Risk, with a clear clinical benefit with postoperative RT. In contrast, over one quarter of high-risk CP patients were reclassified as DS Low Risk with no significant benefit to RT. Thus, for optimizing de-escalation/escalation treatment strategies for DCIS, this analysis further supports that the 7-gene biosignature more reliably discriminates a low-risk group without significant RT benefit compared with CP factors alone and an elevated risk group that benefited from RT, facilitating improved shared decision making.

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023;73:17-48.
2. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. *JAMA Oncol* 2015;1:888-896.
3. Early Breast Cancer Trialists' Collaborative Group (EBCTCG)Correa C, McGale P, et al. Overview of the randomized trials of radiotherapy in ductal carcinoma in situ of the breast. *J Natl Cancer Inst Monogr* 2010;2010:162-177.

4. Wright JL, Gray R, Rahbar H, et al. Lumpectomy without radiation for ductal carcinoma in situ of the breast: 20-year results from the ECOG-ACRIN E5194 study. *NPJ Breast Cancer* 2024;10:16.
5. McCormick B, Winter KA, Woodward W, et al. Randomized phase III trial evaluating radiation following surgical excision for good-risk ductal carcinoma in situ: Long-term report from NRG oncology/RTOG 9804. *J Clin Oncol* 2021;39:3574-3582.
6. Wong JS, Chen YH, Gadd MA, 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;143:343-350.
7. Gradishar WJ, Moran MS, Abraham J, et al. Breast cancer, version 3.2024, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2024;22:331-357.
8. Silverstein MJ, Lagios MD. Choosing treatment for patients with ductal carcinoma in situ: Fine tuning the University of Southern California/Van Nuys Prognostic Index. *J Natl Cancer Inst Monogr* 2010;2010:193-196.
9. Rudloff U, Jacks LM, Goldberg JI, et al. Nomogram for predicting the risk of local recurrence after breast-conserving surgery for ductal carcinoma in situ. *J Clin Oncol* 2010;28:3762-3769.
10. Sweldens C, Peeters S, van Limbergen E, et al. Local relapse after breast-conserving therapy for ductal carcinoma in situ: A European single-center experience and external validation of the Memorial Sloan-Kettering Cancer Center DCIS nomogram. *Cancer J* 2014;20:1-7.
11. Yi M, Meric-Bernstam F, Kuerer HM, 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;30:600-607.
12. Oses G, Mension E, Pumarola C, et al. Analysis of local recurrence risk in ductal carcinoma in situ and external validation of the Memorial Sloan Kettering Cancer Center nomogram. *Cancers (Basel)* 2023;15:2392.
13. Kunkiel M, Niwińska A. Assessment of the usefulness of prognostic Van Nuys Prognostic Index in the treatment in ductal carcinoma in situ in 15-year observation. *Sci Rep* 2021;11:22645.
14. Rakovitch E, Nofech-Mozes S, Hanna W, 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;152:389-398.
15. Bremer T, Whitworth PW, Patel R, et al. A biological signature for breast ductal carcinoma *in situ* to predict radiotherapy benefit and assess recurrence risk. *Clin Cancer Res* 2018;24:5895-5901.
16. Dabbs D, Mittal K, Heineman S, et al. Analytical validation of the 7-gene biosignature for prediction of recurrence risk and radiation therapy benefit for breast ductal carcinoma in situ. *Front Oncol* 2023;13:1069059.
17. Vicini FA, Mann GB, Shah C, et al. A novel biosignature identifies patients with DCIS with high risk of local recurrence after breast conserving surgery and radiation therapy. *Int J Radiat Oncol Biol Phys* 2023;115:93-102.
18. Wärnberg F, Karlsson P, Holmberg E, et al. Prognostic risk assessment and prediction of radiotherapy benefit for women with ductal carcinoma in situ (DCIS) of the breast, in a randomized clinical trial (SweDCIS). *Cancers* 2021;13:6103.
19. Weinmann S, Leo MC, Francisco M, et al. Validation of a ductal carcinoma in situ biomarker profile for risk of recurrence after breast-conserving surgery with and without radiotherapy. *Clin Cancer Res* 2020;26:4054-4063.
20. Shah C, Bremer T, Cox C, et al. Correction to: The clinical utility of DCISionRT® on radiation therapy decision making in patients with ductal carcinoma in situ following breast-conserving surgery. *Ann Surg Oncol* 2021;28:5974-5984.
21. Shah C, Whitworth P, Vicini FA, et al. The clinical utility of a 7-gene biosignature on radiation therapy decision making in patients with ductal carcinoma in situ following breast-conserving surgery: An updated analysis of the DCISionRT® PREDICT study. *Ann Surg Oncol* 2024;31:5919-5928.
22. Benson JR, Wishart GC. Predictors of recurrence for ductal carcinoma in situ after breast-conserving surgery. *Lancet Oncol* 2013;14:e348-e357.
23. Visser LL, Groen EJ, van Leeuwen FE, Lips EH, Schmidt MK, Wesseling J. Predictors of an invasive breast cancer recurrence after DCIS: A systematic review and meta-analyses. *Cancer Epidemiol Biomarkers Prev* 2019;28:835-845.
24. Shumway DA, McLeod CM, Morrow M, et al. Patient experiences and clinician views on the role of radiation therapy for ductal carcinoma in situ. *Int J Radiat Oncol Biol Phys* 2018;100:1237-1245.
25. Punglia RS, Cronin AM, Uno H, et al. Association of regional intensity of ductal carcinoma in situ treatment with likelihood of breast preservation. *JAMA Oncol* 2017;3:101-104.
26. Silver B, Mattessich S, Yacoub I, Rhee B, Schreiber D. Patterns of care and utilization of radiation for women with good-risk ductal carcinoma in situ: A national cancer database analysis. *Cureus* 2022;14:e28223.
27. Waljee JF, Hu ES, Newman LA, Alderman AK. Predictors of re-excision among women undergoing breast-conserving surgery for cancer. *Ann Surg Oncol* 2008;15:1297-1303.
28. Silverstein MJ. The University of Southern California/Van Nuys prognostic index for ductal carcinoma in situ of the breast. *Am J Surg* 2003;186:337-343.
29. G. Mann, O.D. Malley, A. Park, et al., DCIS biologic risk signature predicts risk of recurrence and RT benefit after BCS, *SSO Abstracts*, Accessed August 12, 2025. https://preludedx.com/wp-content/uploads/2021/06/2021-Society-of-Surgical-Oncology-Abstract.pdf.
30. Worni M, Akushevich I, Greenup R, et al. Trends in treatment patterns and outcomes for ductal carcinoma in situ. *J Natl Cancer Inst* 2015;107:djv263.
31. Schmitz RSJM, van den Belt-Dusebout AW, Clements K, et al. Association of DCIS size and margin status with risk of developing breast cancer post-treatment: Multinational, pooled cohort study. *BMJ* 2023;383:e076022.
32. van Seijen M, Jóźwiak K, Pinder SE, et al. Variability in grading of ductal carcinoma in situ among an international group of pathologists. *J Pathol Clin Res* 2021;7:233-242.
33. Thompson AM, Clements K, Cheung S, et al. Management and 5-year outcomes in 9938 women with screen-detected ductal carcinoma in situ: The UK Sloane Project. *Eur J Cancer* 2018;101:210-219.
34. Pinder SE, Duggan C, Ellis IO, et al. A new pathological system for grading DCIS with improved prediction of local recurrence: Results from the UKCCCR/ANZ DCIS trial. *Br J Cancer* 2010;103:94-100.
35. Tadros AB, Smith BD, Shen Y, et al. Ductal carcinoma in situ and margins <2 mm: Contemporary outcomes with breast conservation. *Ann Surg* 2019;269:150-157.
36. Vicini FA, Kestin LL, Goldstein NS, Baglan KL, Pettinga JE, Martinez AA. Relationship between excision volume, margin status, and tumor size with the development of local recurrence in patients with ductal carcinoma-in-situ treated with breast-conserving therapy. *J Surg Oncol* 2001;76:245-254.
37. Shah C, Wobb J, Manyam B, et al. Management of ductal carcinoma in situ of the breast: A review. *JAMA Oncol* 2016;2:1083-1088.
38. Staley H, McCallum I, Bruce J. Postoperative tamoxifen for ductal carcinoma in situ: Cochrane systematic review and meta-analysis. *Breast* 2014;23:546-551.
39. Solin LJ, Fourquet A, Vicini FA, et al. Long-term outcome after breast-conservation treatment with radiation for mammographically detected ductal carcinoma in situ of the breast. *Cancer* 2005;103:1137-1146.
40. I. Wapnir, R. Cecchini, J. Dignam, et al., Margin width and local recurrence in the NRG oncology/NSABP B-35 DCIS lumpectomy trial, *Am Soc Breast Surgeons Annual Meeting Presentation*, **B-35**, Accessed August 12, 2025. https://www.breastsurgeons.org/meeting/2025/docs/ASBrS-2025-Press-Release-Margins-Wapnir.pdf.
41. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: Report of the national surgical adjuvant breast and bowel project P-1 study. *J Natl Cancer Inst* 1998;90:1371-1388.
42. Wapnir IL, Dignam JJ, Fisher B, 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;103:478-488.

43. Whitworth PW, Shah CS, Vicini FA, et al. Assessing the benefit of adjuvant endocrine therapy in patients following breast-conserving surgery with or without radiation stratified by a 7-gene predictive DCIS biosignature. *J Clin Oncol* 2022;40:502.

44. Cuzick J, Sestak I, Pinder SE, 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;12:21-29.