original reports

Clinicogenomic Radiotherapy Classifier Predicting the Need for Intensified Locoregional Treatment After Breast-Conserving Surgery for Early-Stage Breast Cancer

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abstract

PURPOSE Most patients with early-stage breast cancer are treated with adjuvant radiotherapy (RT) after breast-conserving surgery (BCS) to prevent locoregional recurrence (LRR). However, no genomic tools are used currently to select the optimal RT strategy.

METHODS We profiled the transcriptome of primary tumors on a clinical grade assay from the SweBCG91-RT trial, in which patients with node-negative breast cancer were randomly assigned to either whole-breast RT after BCS or no RT. We derived a new classifier, Adjuvant Radiotherapy Intensification Classifier (ARTIC), comprising 27 genes and patient age, in three publicly available cohorts, then independently validated ARTIC for LRR in 748 patients in SweBCG91-RT. We also compared previously published genomic signatures for ability to predict benefit from RT in SweBCG91-RT.

RESULTS ARTIC was highly prognostic for LRR in patients treated with RT (hazard ratio [HR], 3.4; 95% CI, 2.0 to 5.9; $P < .001$) and predictive of RT benefit ($P_{interaction} = .005$). Patients with low ARTIC scores had a large benefit from RT (HR, 0.33 [95% CI, 0.21 to 0.52], $P < .001$; 10-year cumulative incidence of LRR, 6% *v* 21%), whereas those with high ARTIC scores benefited less from RT (HR, 0.73 [95% CI, 0.44 to 1.2], $P = .23$; 10-year cumulative incidence of LRR, 25% *v* 32%). In contrast, none of the eight previously published signatures were predictive of benefit from RT in SweBCG91-RT.

CONCLUSION ARTIC identified women with a substantial benefit from RT as well as women with a particularly elevated LRR risk in whom whole-breast RT was not sufficiently effective and, thus, in whom intensified treatment strategies such as tumor-bed boost, and possibly regional nodal RT, should be considered. To our knowledge, ARTIC is the first classifier validated as predictive of benefit from RT in a phase III clinical trial with patients randomly assigned to receive or not receive RT.

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ASSOCIATED CONTENT

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Data Supplement

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INTRODUCTION

Adjuvant whole-breast radiotherapy (RT) after breast-conserving surgery (BCS) is standard of care for women with early-stage invasive breast cancer for local management of their disease. RT provides a significant reduction in locoregional recurrence (LRR) risk and increased breast cancer–specific survival. However, meta-analyses from the Early Breast Cancer Trialists' Collaborative Group have shown that at 10 years after surgery, 10% of patients with node-negative disease will still experience a local recurrence after BCS, even with the receipt of RT.[1,2] The addition of an RT boost to the tumor bed reduces the risk of local recurrence, but it is potentially associated with more toxicity, and most patients will remain free from local recurrence even without the boost.[3] Several clinicopathologic risk factors are predictive of increased local recurrence rates after BCS and whole-breast RT, including young age, high histologic grade, positive margins, and vascular invasion, but the relative effect of RT has been constant over these groups.[2,4-8] Accordingly, clinical guidelines are based on baseline risk and emphasize the importance of achieving negative margins in all patients treated with breast-conserving therapy and recommend the use of an RT boost to the tumor bed for patients of young age and/or clinically defined high-risk tumors. The guidelines, however, do not incorporate genomic information for tumor radiosensitivity, and clinical practice varies widely between regions.[6,9] Although the use of regional RT is not

generally recommended for patients with node-negative disease, some patients with centrally or medially located tumors or high-risk phenotypes may benefit from receiving regional RT.[10,11] Clearly there is a need to better identify patients up front who will benefit from whole-breast RT with standard doses and those who are at increased risk for a LRR despite conventional treatment. For this latter group, a more tailored locoregional approach could improve outcomes.

Although breast cancer subtypes and previous genomic classifiers developed either to predict distant metastasis,[12-19] or specifically to predict locoregional recurrence,[20-27] have shown promise to prognosticate local recurrence, the ability of these classifiers to guide RT decisions has yet to be tested, to our knowledge, in a trial randomized for RT. Thus, their introduction in clinical practice awaits definitive demonstration of clinical utility in large, clinical trials in which patients are randomly assigned to receive RT or not and, ultimately, in prospective clinical trials, per national and international guideline recommendations.[28]

In the present study, we aimed to use publicly available data sets to develop a gene expression–based classifier that could predict benefit from RT and guide clinical use of RT intensification strategies. For validation of the novel classifier, we performed transcriptomic profiling of data from the SweBCG91-RT trial on a clinical-grade platform. The SweBCG91-RT trial was a multi-institutional trial in which women with stage I-II, node-negative breast cancer were randomly assigned to postoperative whole-breast RT or no RT after BCS with negative margins.[19,29] In the trial, all subsets of patients, based on clinical risk factors, benefitted from RT.[8] To our knowledge, this is the first gene expression data set from a trial in which patients were randomly assigned to receive RT or no RT after BCS. This data set allowed us to validate our classifier for prediction of benefit from RT, as well as contrast its performance with existing genomic signatures.

METHODS

Training Data Sets for Classifier

To develop a radiotherapy intensification classifier, we used three publicly available, early-stage breast cancer gene-expression data sets from patients treated with RT and with detailed local recurrence information. These expression data sets were the Servant data set,[22] the van de Vijver data set,[30] and the Sjöström data set.[25] Details are provided in the Data Supplement.

SweBCG91-RT Validation Data Set

We performed gene expression analysis of the SweBCG91-RT trial, the details of which have been published.[8,19,29] Briefly, the 1,178 patients with node-negative, stage I-IIA disease who were undergoing BCS were randomly assigned to adjuvant whole-breast RT or no RT. All patients had negative surgical margins. For patients in the RT arm, a boost to the tumor bed was not provided. Systemic adjuvant therapy was administered according to regional guidelines at the time and was sparsely used. Subtyping was performed using immunohistochemical staining of ER, PgR, HER2, and Ki67, as previously described.[19] The median follow-up was 15.0 years for LRR in patients free from event. The trial and follow-up study were approved by the Regional Ethical Review Board at Lund University (approval numbers 2010/127 and 2015/548). Informed oral consent was obtained from all patients. We followed Reporting Recommendations for Tumor Marker Prognostic Studies guidelines for reporting of this study.[31]

Formalin-fixed, paraffin-embedded tissue samples from 922 primary tumors of SweBCG91-RT were available for additional processing, of which 748 had sufficient RNA, passed quality control, and were included in the final analysis (Data Supplement). Gene expression data were acquired from GeneChip Human Exon 1.0 ST Arrays (Thermo Fisher Scientific, South San Francisco, CA). Data are available at Gene Expression Omnibus with accession number GSE119295. For more details, see the Data Supplement. The tumors included for gene expression analysis were marginally larger and slightly less likely to be of Luminal A subtype and of histologic grade 1 (Data Supplement).

Computation of Previously Published Breast Cancer Risk Scores

From the literature, we identified eight previously published genomic signatures developed for radiation sensitivity, for local recurrence and/or LRR, or for distant recurrence, and that then subsequently were evaluated for ability to prognosticate LRR.[12,13,17,21,24-27] We applied gene expression data from the GeneChip Human Exon 1.0 ST microarrays to genomic signature equations to calculate continuous risk scores using the equations as defined in the original publications (Data Supplement).

Statistical Methods

Statistical analyses were performed using R, version 3.5.2 (https://cran.r-project.org/bin/windows/base/old/3.5.2/). The author responsible for deriving the classifier described here was blinded to the full SweBCG91-RT cohort. Validation of the classifier was performed by an author not involved in classifier development (Data Supplement). The primary end point was cumulative incidence of LRR using time to LRR as first event. Cumulative incidences were computed using a competing risk approach (R cmprsk package).[32] Distant metastasis and death without recurrence were considered competing events. Eight patients had synchronous distant metastasis and LRR, defined as LRR registered at the same time or within 3 months as the metastasis, and they were regarded as having an LRR. Cause-specific Cox proportional hazards regression models were used to infer the relationship of genomic and clinical variables on LRR, and *P* values for differences between groups were calculated with the Wald test or the equivalent

log-rank test. The proportional hazards assumption was checked graphically and with the Schoenfeld test. It was violated ($P < .05$) for the interaction model of Adjuvant Radiotherapy Intensification Classifier (ARTIC; Table 1), RT in the full cohort, subtype, histologic grade, and for several published signatures. In addition, RT and prognostic signatures had a larger HR during the first 5 years. Thus, the presented hazard ratios (HRs) should be interpreted as the mean over the follow-up period. HRs are reported with 95% CIs.

Classifier scores were tested as dichotomized to high/low, except when testing for interaction between classifier/signature score and RT, and for the genomic-only model analysis, where the continuous score was used, as recommended.[33] For dichotomization, we applied a prespecified threshold at the 75th percentile for all signature scores, based on the rate of LRR from the Early Breast Cancer Trialists' Collaborative Group meta-analysis where approximately 25% of patients with early, node-negative breast cancer experienced an LRR without RT. This choice of threshold was made deliberately to evaluate ARTIC and previous classifiers in a similar way, because scores from published signatures are calculated on other platforms, and several of the original classifier thresholds are not specifically optimized for LRR. All tests for interaction were performed using continuous signature scores; thus, they were not dependent on any threshold.

RESULTS

ARTIC Independently Validated as Predictive of Benefit From RT

We derived the ARTIC, a clinicogenomic classifier comprising the expression of 27 genes and patient age, in publicly available cohorts treated with RT (Data Supplement). Model gene selection was performed in the training data set. Because age was the strongest clinical factor for the end point in the training data set, and in practice can be consistently and easily obtained for every patient, it was included as a model variable (Data Supplement). We then independently validated ARTIC in the SweBCG91-RT cohort. Patients with low ARTIC scores had one-third the risk of LRR with RT (HR, 0.33; 0.21 to 0.52; $P < .001$ for RT arm compared with no-RT arm). At 10 years, there was a 15% absolute reduction in incidence of LRR for patients who received RT compared with those who did not (10-year cumulative incidence of LRR for patients with low classifier scores who did not receive RT, 0.21; for those who did receive RT, 10-year cumulative incidence of LRR was 0.06; Fig 1A). However, patients with high ARTIC scores had less benefit from whole-breast RT (HR, 0.73 [0.44 to 1.2], $P = .23$; 10-year cumulative incidence of LRR for patients with high scores who did not receive RT, 0.32 and 0.25 for those who did receive RT; Fig 1B). The classifier was predictive of RT benefit ($P_{interaction} = .005$; Fig 1C; Table 1). The interaction remained statistically significant in

TABLE 1. ARTIC Interaction With Radiation Therapy in the SweBCG91-RT Validation Cohort

Interaction	HR (95% CI)*	P
Interaction of ARTIC and RT by UVA		
ARTIC	1.8 (0.98 to 3.3)	.059
RT	0.058 (0.013 to 0.26)	< .001
ARTIC:RT	4.2 (1.5 to 12)	.0051
Interaction of ARTIC and RT adjusted for clinical variables by MVA		
ARTIC	1.8 (0.96 to 3.4)	.066
RT	0.065 (0.014 to 0.3)	< .001
Subtype by IHC		
Luminal B (HER2−)	1.1 (0.75 to 1.6)	.64
Triple negative	1 (0.54 to 2)	.92
HER2+	1.2 (0.63 to 2.2)	.59
Histologic grade		
2	1.8 (0.99 to 3.2)	.053
3	2 (1 to 3.8)	.048
Size ≥ 20 mm	0.37 (0.16 to 0.84)	.018
Systemic therapy	1.1 (0.67 to 1.9)	.65
ARTIC:RT	4 (1.4 to 11)	.0076

NOTE. Classifier is continuous.

Abbreviations: ARTIC, Adjuvant Radiotherapy Intensification Classifier; HR, hazard ratio; IHC, immunohistochemistry; MVA, multivariable analysis; RT, radiotherapy; UVA, univariable analysis.

*UVA HRs reported for interaction of ARTIC and RT, and MVA HRs reported for interaction of ARTIC and RT adjusted for clinical variables

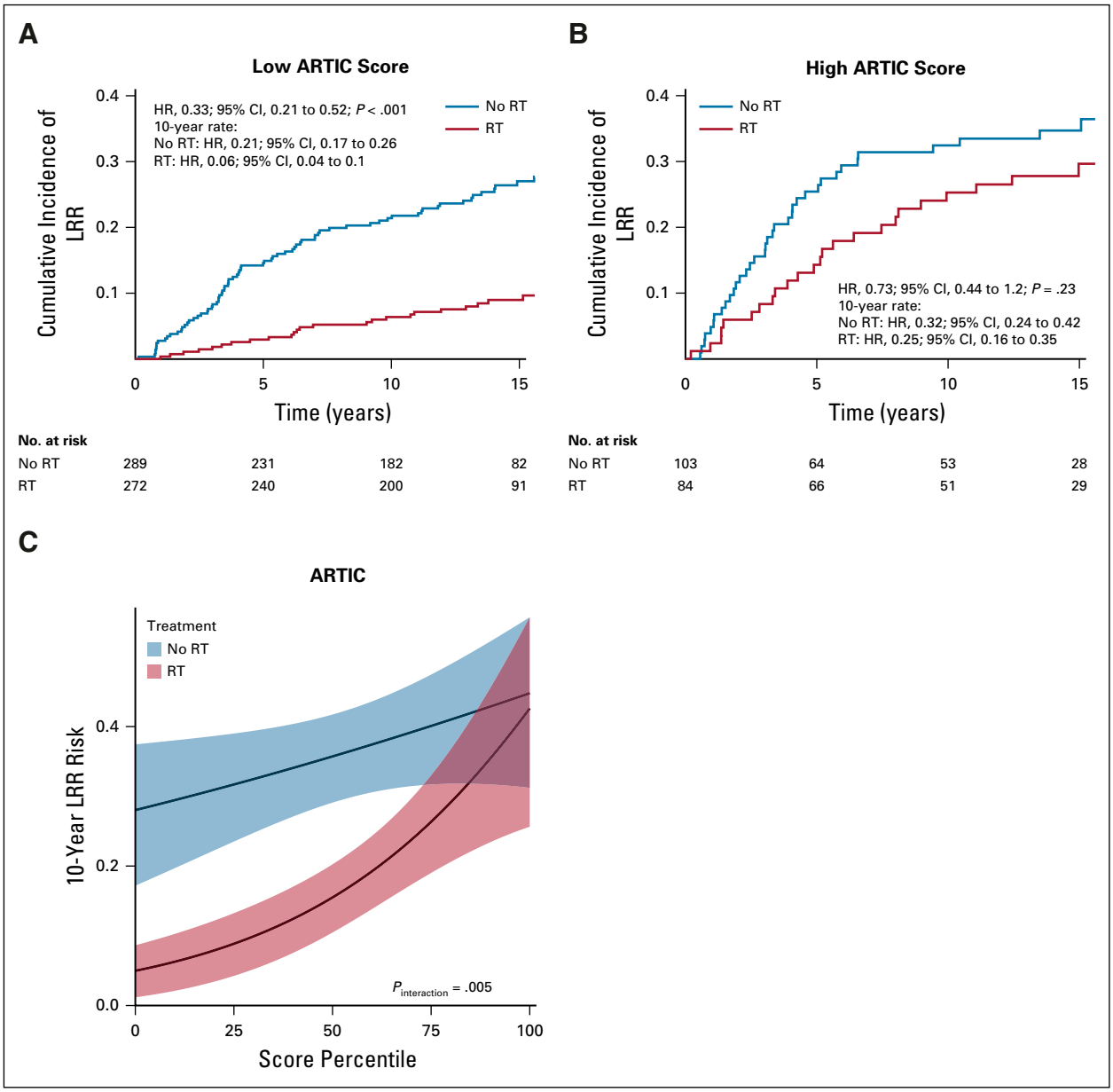

FIG 1. Performance of Adjuvant Radiotherapy Intensification Classifier (ARTIC) for prognostication of locoregional recurrence (LRR) and treatment prediction for adjuvant radiotherapy (RT) in the SweBCG91-RT validation cohort. Cumulative incidence of LRR for high and low classifier scores (as split by the 75th percentile score) and interaction with RT. (A) RT benefit in patients classified as low risk by ARTIC. (B) RT benefit in patients classified as high risk by ARTIC. (C) Interaction of RT and ARTIC. Continuous classifier scores are presented with the risk for LRR with or without RT. The 10-year LRR-free interval risk was calculated by fitting a cause-specific Cox regression model to time to LRR using the interaction of calculated ARTIC scores and RT status. Predicted survival curves and variances were generated using the Efron approach and the CIs were constructed using the log approach.[34] HR, hazard ratio.

multivariable analysis (MVA) including systemic therapy, subtype, tumor size, and histologic grade (P = .008; Table 1). ARTIC was highly prognostic for LRR in patients treated with RT (HR, 3.4; 2.0 to 5.9; P < .001) in univariable analysis (UVA; Fig 2; Table 2) and remained prognostic in MVA (HR, 3.4; 1.9 to 6.0; P < .001; Table 2). In patients not treated with RT, ARTIC was also prognostic in both UVA and MVA (UVA HR, 1.6 [1.0 to 2.3], P = .028; MVA HR, 1.5 [1 to 2.3], P = .041; Table 2).

Elevated Local and Regional Recurrences in Patients With High ARTIC Scores

ARTIC identified patients at elevated risk of local and regional recurrences despite receiving whole-breast RT (Table 3). The majority of local recurrences (90%; Table 3) occurred in the same quadrant as the primary tumor. Among patients treated with whole-breast RT, 20 of 84 (23.8%) of those with high ARTIC scores experienced a local recurrence, whereas 25 of 272 (9.2%) of those with

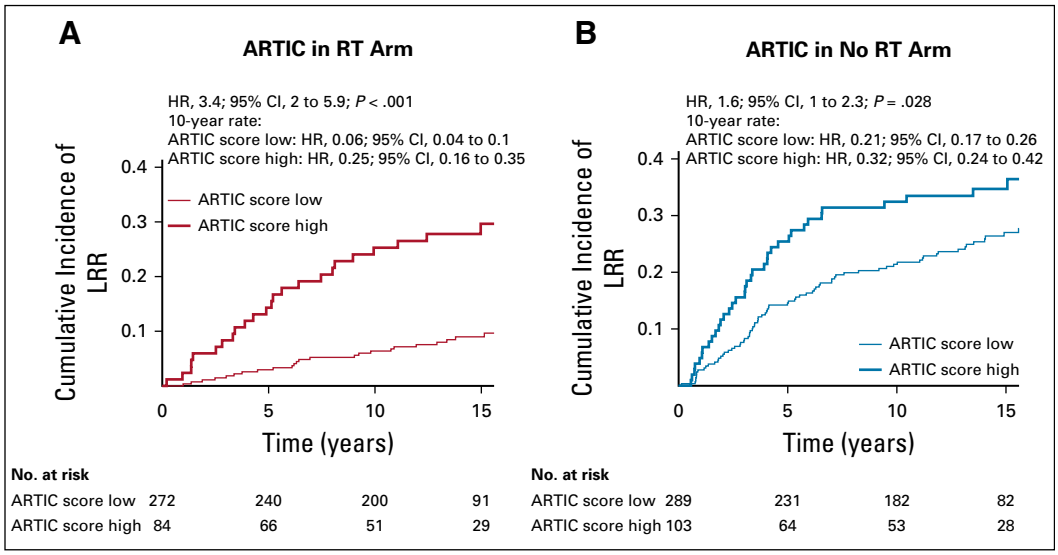

FIG 2. Prognostic performance of Adjuvant Radiotherapy Intensification Classifier (ARTIC) in the SweBCG91-RT validation cohort. Cumulative incidence of locoregional recurrence (LRR) for patients split by the 75th percentile score in (A) the radiotherapy (RT)-treated arm and (B) the no RT arm. HR, hazard ratio.

low scores experienced a local recurrence ($P < .001$). Furthermore, among patients treated with whole-breast RT, nine of 84 (10.7%) of those with high ARTIC scores experienced a regional recurrence, whereas one of 272 (0.4%) of those with low scores experienced a regional recurrence ($P < .001$). The majority of regional recurrences occurred in the axilla (84.6%). The effect of RT was more pronounced among the patients with low ARTIC score both for local recurrence and regional recurrences (local recurrence: 9.2% *v* 22.8%, $P < .001$; regional recurrence: 0.4% *v* 4.8%; $P = .001$, for patients treated with RT compared with no RT, respectively), compared with those with high ARTIC score (local recurrence: 23.8% *v* 34.0%, $P = .05$; regional recurrence: 10.7% *v* 5.8%, $P = .3$). The interaction of ARTIC with RT was significant for regional recurrences ($P = .001$), and a trend was observed for local recurrences ($P = .1$).

Patient Age and Gene-Expression Information Are Complementary

We next sought to confirm that the prognostic and predictive performances of ARTIC are driven by the inclusion of both the genomic data and patient age. We examined these components individually for prognostic ability for LRR and predictive ability for RT. The genomic portion of the model, evaluated as a model of 27 genes, was both prognostic for LRR (HR, 3.5; 95% CI, 1.7 to 7.3; $P = .001$) and predictive ($P_{interaction} = .024$) for RT benefit in the SweBCG91-RT cohort (Data Supplement). Age was also prognostic (HR, 0.97; 95% CI, 0.96 to 0.99; $P = .0026$) and predictive ($P_{interaction} = .025$; Data Supplement). The inclusion of the genomic portion of the model and age in a Cox regression model revealed that both were statistically significant to the LRR end point (age, $P = .002$; genomic, $P = .001$), indicating that the two provided independent information from each other in the final ARTIC model (Data Supplement), justifying inclusion of both components in the final classifier.

No Previously Published Signatures Have a Significant Interaction With RT in the SweBCG91-RT Cohort

Many gene signatures have been published that purportedly predict LRR in early-stage breast cancer.[12,13,17,20-27] We calculated and examined eight previously published signatures in SweBCG91-RT (Fig 3). Two of the eight signatures, the top scoring pairs (TSP) intensification, and the 70-gene signatures, were prognostic in the patients receiving RT ($P < .05$) for the LRR end point in univariable Cox regression analysis (Fig 3). Three of eight signatures (the TSP intensification, the TSP omission, and the 70-gene signature) were prognostic ($P < .05$) in multivariable analysis that included chemotherapy, endocrine therapy, subtype, tumor size, and histologic grade (Data Supplement) in the model. However, none of the previously published signatures had a significant interaction with RT ($P < .05$), and the effect of RT was consistent for high and low scores (Fig 3; Data Supplement).

DISCUSSION

Despite adjuvant whole-breast RT with standard tangents, a significant proportion of women with early-stage breast cancer treated with BCS will still suffer an LRR.[1,2] In this study, we developed and independently validated a clinicogenomic classifier, ARTIC, which identifies patients who derive a significant benefit from adjuvant RT and those at high risk of LRR with less pronounced benefit from standard radiation doses. In the SweBCG91-RT randomized control trial, the ARTIC classifier identified a subset of patients with early-stage (ie, node-negative, stage I-IIA)

TABLE 2. Prognostic Performance of ARTIC in the SweBCG91-RT Validation Cohort

	UVA		MVA	
Arm	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
RT				
ARTIC	3.4 (2 to 5.9)	< .001	3.4 (1.9 to 6)	< .001
Subtype by IHC				
Luminal B (HER2−)	0.66 (0.33 to 1.3)	.24	0.59 (0.29 to 1.2)	.15
Triple negative	0.82 (0.25 to 2.7)	.74	0.6 (0.16 to 2.2)	.44
HER2+	1.2 (0.45 to 3)	.76	1.1 (0.36 to 3.2)	.89
Histologic grade				
2	1.8 (0.71 to 4.7)	.21	1.7 (0.66 to 4.4)	.27
3	2.4 (0.84 to 6.6)	.1	2 (0.64 to 6.2)	.24
Size ≥ 20 mm	1.2 (0.55 to 2.7)	.63	1.4 (0.59 to 3.3)	.45
Systemic therapy	0.52 (0.13 to 2.1)	.37	0.37 (0.079 to 1.8)	.22
No RT				
ARTIC	1.6 (1 to 2.3)	.028	1.5 (1 to 2.3)	.041
Subtype by IHC				
Luminal B (HER2−)	1.5 (0.96 to 2.2)	.08	1.4 (0.87 to 2.1)	.18
Triple negative	1.5 (0.76 to 2.8)	.26	1.4 (0.67 to 3)	.37
HER2+	1.6 (0.8 to 3.3)	.18	1.2 (0.56 to 2.7)	.6
Histologic grade				
2	1.8 (0.87 to 3.8)	.11	1.7 (0.8 to 3.5)	.17
3	2.3 (1.1 to 4.9)	.036	1.9 (0.82 to 4.4)	.13
Size ≥ 20 mm	0.78 (0.45 to 1.4)	.39	0.98 (0.51 to 1.9)	.94
Systemic therapy	0.41 (0.17 to 1)	.052	0.37 (0.13 to 1)	.05

NOTE. Classifier is dichotomized.

Abbreviations: ARTIC, Adjuvant Radiotherapy Intensification Classifier; HR, hazard ratio; IHC, immunohistochemistry; MVA, multivariable analysis; RT, radiotherapy; UVA, univariable analysis.

disease with an elevated risk of LRR after BCS with negative margins, even with whole-breast RT. To our knowledge, this is the first classifier validated as both prognostic for LRR and predictive for benefit from RT in a large, phase III trial in which patients were randomly assigned to receive RT or not.

Treatment intensification options are available for these patients at high risk of LRR despite standard whole-breast RT. The addition of a tumor-bed boost improves rates of local recurrence.[3] However, patient selection for use of a boost is based on broad clinical criteria such as age and tumor risk factors and does not incorporate genomic information.[6,9] Given that 88% of the local recurrences in the ARTIC high-risk group occurred in the same quadrant as the primary tumor, many of the patients who experienced a local recurrence would likely have benefitted from a boost. Thus, ARTIC may represent a tool to identify patients who may benefit from a tumor-bed boost based on the molecular characteristics of the tumor.

Furthermore, ARTIC high-risk tumors had an elevated risk of regional recurrence, predominantly in the axilla, whereas ARTIC low-risk tumors had low rates of regional recurrence. In EORTC 22922 (ClinicalTrials.gov identifier: NCT00002851), in which patients undergoing BCS or mastectomy with axillary dissection were randomly assigned to whole-breast/chest wall RT with or without regional nodal RT, 44% of patients were node negative and, in the subgroup analysis, patients with node-negative disease seemed to benefit from regional treatment.[10] Similarly, in MA.20, in which patients undergoing BCS and axillary dissection or sentinel node biopsy were randomly assigned to whole-breast RT with or without regional RT, 10% were node negative with high-risk tumors, and this subgroup of patients seemed to benefit from regional RT.[11] It should be noted that the majority of node-negative assessments in the EORTC 22922, MA.20, and in the SweBCG91-RT trials were defined on the basis of axillary lymph node dissection, which is likely less sensitive to small-volume lymph node metastases compared with sentinel lymph node biopsy specimens. Regional nodal RT does increase toxicity,[10,11,35] and the administration to patients with node-negative disease would require careful patient selection. The rates of regional recurrences in

TABLE 3. Patterns of Recurrence

	Total	ARTIC Score Low		ARTIC Score High	
Event		**No RT**	**RT**	**No RT**	**RT**
No.	748	289	272	103	84
Any locoregional recurrence	163 (21.8)	76 (26.3)	26 (9.6)	36 (35)	25 (29.8)
Local recurrence	146 (19.5)	66 (22.8)	25 (9.2)	35 (34)	20 (23.8)
Local recurrence in the same quadrant					
Yes	72 (90)	36 (94.7)	8 (80)	20 (90.9)	8 (80)
No	8 (10)	2 (5.3)	2 (20)	2 (9.1)	2 (20)
Missing	66	28	15	13	10
Regional recurrence	30 (4)	14 (4.8)	1 (0.4)	6 (5.8)	9 (10.7)
In the axilla	22 (84.6)	12 (85.7)	0 (0)	5 (100)	5 (71.4)
In the supraclavicular fossa	3 (11.5)	2 (14.3)	0 (0)	0 (0)	1 (14.3)
Simultaneously in axilla and supraclavicular fossa	1 (3.8)	0 (0)	0 (0)	0 (0)	1 (14.3)
Missing detailed site information	4	0	1	1	2
Both local and regional recurrence	13 (1.7)	4 (1.4)	0 (0)	5 (4.9)	4 (4.8)
Distant metastasis, first event	60 (8)	15 (5.2)	22 (8.1)	11 (10.7)	12 (14.3)
Distant metastasis	106 (14.2)	36 (12.5)	26 (9.6)	25 (24.3)	19 (22.6)
Breast cancer death*	136 (18.2)	50 (17.3)	40 (14.7)	25 (24.3)	21 (25)
Death without breast cancer	193 (25.8)	89 (30.8)	84 (30.9)	12 (11.7)	8 (9.5)
Death from any cause*	355 (47.5)	151 (52.2)	133 (48.9)	40 (38.8)	31 (36.9)

NOTE. Data reported as No. (%) unless otherwise indicated.

*Breast cancer death/death without breast cancer and death from any cause have longer follow-up because these data were extracted from the cause of death registry and death registry, respectively, whereas recurrence follow-up formation data are from patient charts. Median follow-up times for patients free from event are as follows: any locoregional recurrence, 15.0 years; distant metastasis, 15.1 years; breast cancer death/death without breast cancer, 20.0 years; death from any cause, 21.2 years.

EORTC 22922 and MA.20 (2.7% and 0.5% in the regional RT arm, respectively) are similar to the patients with low ARTIC scores in the RT arm (0.4%) but substantially higher in the patients with high ARTIC scores (10.7%). The regional recurrences in this study were predominantly axillary, and this classifier may represent a way of selecting patients with node-negative disease who would benefit from nodal RT, perhaps focused on the axilla, based on the molecular characteristics of the tumor, especially in an era of less aggressive axillary surgery. However, the absolute number of regional recurrences was low, and the results need additional validation.

Few patients in this study were treated with adjuvant systemic therapy, which is one explanation for the high rate of LRR observed. This low rate of systemic therapy use contrasts with current management of invasive breast cancer in which chemotherapy and/or endocrine therapy is broadly used, although the adherence to these treatments may be as low as 50%,[36] emphasizing the role of RT in local control. Patients with high ARTIC scores should be offered appropriate systemic therapy in addition to RT, and the absolute risk of LRR would be lower in a modern-day setting than that presented herein. On the other hand, the use of RT as a single adjuvant therapy for most patients in this study gave us a unique opportunity to validate a classifier specific for RT benefit without confounding by systemic therapy, and we believe our classifier could aid a more tailored selection of patients for intensified RT treatment. Furthermore, although ARTIC is highly prognostic and predictive in multivariable models including known risk factors, clearly showing independent information, there is still an association of high ARTIC scores with clinical risk factors, such as high histologic grade and triple-negative or HER2+ subtypes (Data Supplement). Taken together, studies aimed at validating ARTIC in patients treated with systemic therapy, and the integration with clinical risk factors, will guide eventual clinical implementation.

Although several signatures purporting to be used for radiation decisions have been published, we found that none were predictive for benefit from RT by interaction analysis, using samples from a phase III, randomized RT trial. Genes in ARTIC include genes related to cell cycle, proliferation, and kinase activity, which are also represented in many of the published signatures. For example, the 70-gene and 21-gene signatures include a strong focus on proliferation.[37,38] Three genes in the ARTIC gene list overlapped with some previously published signatures: *KPNA2* and *BTG3* in the

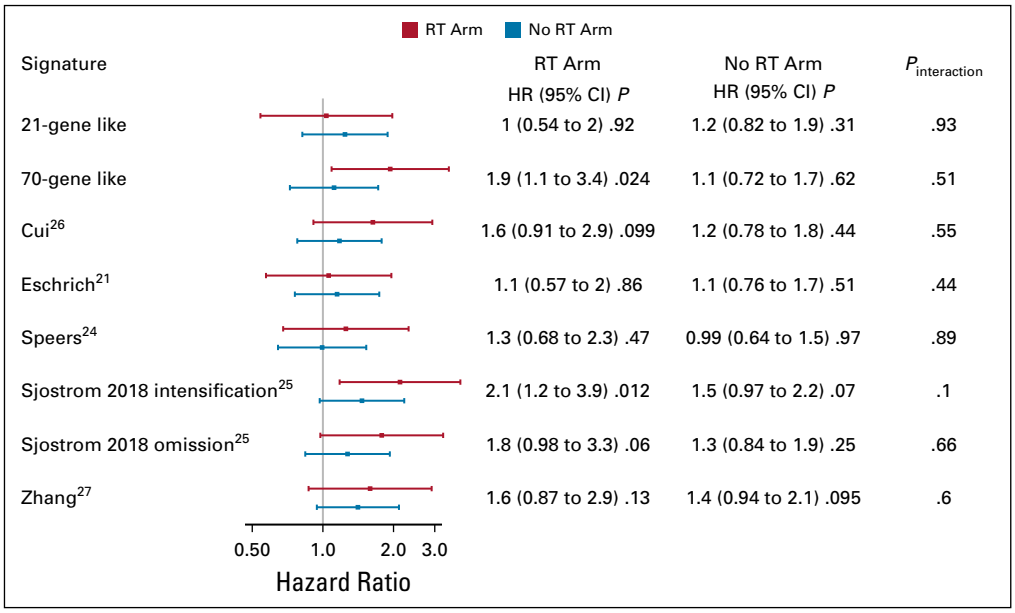

FIG 3. Evaluation of previously-published signatures prognostic for locoregional recurrence and/or treatment predictive for adjuvant radiotherapy (RT) in the SweBCG91-RT validation cohort. Previously published signatures were evaluated with cause-specific Cox proportional hazards modeling in the RT arm and in the no-RT arm. For prognostication, patients were split by the 75th percentile score with the respective signatures. For interaction between RT and signatures, continuous signature scores were used. HR, hazard ratio.

Cui 2018 signature and *CCNB1* in the 21-gene signature.[26,37] However, our signature was trained and selected using three publicly available cohorts with carefully detailed local recurrence information, whereas others were built within cell lines or with metastasis or overall survival end points. Furthermore, we selected for genes that had good technical characteristics in formalin-fixed, paraffin-embedded and fresh frozen tissue, which may explain the improved performance of ARTIC compared with these published signatures.

In conclusion, we developed ARTIC, a radiotherapy intensification classifier, and validated the classifier as highly prognostic for LRR and predictive for benefit from RT in a large, randomized, phase III trial. Specifically, the classifier can identify patients with high-risk, node-negative, stage I-IIA breast cancer who have a three-fold higher LRR rate after BCS with whole-breast RT. This subgroup may preferentially benefit from intensification of local treatment including use of a boost and possibly regional nodal radiation.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinicogenomic Radiotherapy Classifier Predicting the Need for Intensified Locoregional Treatment After Breast-Conserving Surgery for Early-Stage Breast Cancer

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