Molecular Predictive and Prognostic Markers in Locoregional Management

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INTRODUCTION

In early-stage breast cancer (BC), molecular subtyping and gene expression profiling have improved our ability to estimate the risk of distant recurrence above and beyond clinicopathologic factors and commonly used biomarkers.[1-6] Several gene expression signatures have been validated to predict risk of distant recurrence both in patients who have received no adjuvant systemic therapy and in those treated with adjuvant hormonal therapy with or without adjuvant chemotherapy.[1,3-13]

Despite the recent progress in refining risk of distant recurrence with genomic classifiers, assessment of risk for locoregional recurrence (LRR) still primarily relies on traditional clinicopathologic factors such as patient age, tumor size, grade, pathologic nodal status, presence of lymphovascular space invasion (LVSI), and margin width.[14,15] In addition, several studies have shown that tumor subtype alone is strongly predictive of LRR.[16-23] Given the strong correlation between the risk of LRR and distant recurrence,[24-26] recent studies have investigated whether genomic assays that predict risk of distant recurrence can also predict risk of local recurrence.[27-35] Increasingly, new genomic classifiers are being developed specific to LRR in patients with node-negative and node-positive invasive BC.[36,37] There is also increasing interest in the development of gene expression assays to predict response to radiation (XRT) therapy.[37-46]

Local recurrence is also strongly influenced by the surgical approach; however, to date, molecular subtyping and genomic profiling have little to no influence on the extent of surgical therapy, which is traditionally based on the anatomic extent of the tumor and not on underlying tumor biology. Anatomic extent of the tumor in the breast and axilla can be reduced by neoadjuvant therapy, with resulting tailoring of the surgical approach.

MOLECULAR AND RECEPTOR-BASED SURROGATE SUBTYPES FOR PREDICTION OF RISK OF LRR

Several studies have examined the association between BC subtypes based on receptor surrogates for gene expression and LRR, and the results are summarized in Table 1. Nguyen et al[16] were among the first to report the association between LRR and BC subtype as determined by immunohistochemistry in patients treated with breast-conserving surgery (BCS) plus breast XRT. They used receptor status to approximate subtype as follows: luminal A—estrogen receptor (ER) and progesterone receptor (PR) positive and human epidermal growth factor receptor 2 (HER2) negative; luminal B—ER, PR, and HER2 positive; HER2—ER and PR negative and HER2 positive; and basal—ER, PR, and HER2 negative. The 5-year cumulative incidence of LRR was 0.8% for luminal A, 1.5% for luminal B, 8.4% for HER2, and 7.1% for basal/triple-negative subtype. On multivariable analysis with luminal A as baseline, HER2 (hazard ratio [HR], 9.2; $P = .012$) and basal (HR, 7.1; $P = .009$) subtypes were associated with increased risk of LRR. On multivariable analysis, luminal B (HR, 2.9; $P = .007$) and basal (HR, 2.3; $P = .035$) subtypes were associated with increased risk of distant metastases. These findings suggest a potentially increased XRT benefit in the luminal B subtype and potential XRT resistance in HER2 and basal subtypes. Of note, these patients were treated in the pretrastuzumab era. A more recent report from the same group on an expanded cohort of patients[17] showed similar findings, with a 5-year cumulative incidence of LRR of 0.8% for luminal A, 2.3% for luminal B, 1.1% for luminal HER2, 10.8% for HER2, and 6.7% for triple negative.

Several other investigators have reported similar findings. Retrospective assessment of molecular phenotypes in an Australian randomized trial of BCS and whole-breast XRT with or without boost reported 10-year LRR rates of 4.8% for luminal A, 8.6% for luminal B, 17.3% for basal, and 15.3% for HER2-positive tumors ($P = .012$).[18] Investigators from the British Columbia Cancer Agency reported 10-year LRR rates with BCS plus XRT of 8% for luminal A and 10% for luminal B tumors, compared with 21% for HER2-enriched and 14% for basal subtypes, which was statistically significant on multivariable analysis.[19] Interestingly, for mastectomy patients, who generally did not receive XRT, luminal B tumors were also associated with an increased risk of LRR on multivariable analysis. The higher LRR for luminal B tumors in patients undergoing mastectomy but not in those undergoing BCS plus XRT suggests that luminal B tumors are sensitive to XRT.

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CONTEXT

Key Objective

To review evidence on the association between breast cancer subtypes/genomic classifiers and locoregional recurrence in early-stage breast cancer.

Knowledge Generated

Several studies have demonstrated an association between breast cancer subtypes and risk of locoregional recurrence. In addition, multiple studies have also demonstrated a strong association between commercially available and non-commercially available genomic classifiers and risk of locoregional recurrence.

Relevance

Although currently available evidence provides great insight on tumor biology and its locoregional behavior, such evidence has not yet translated into meaningful changes in locoregional treatment approaches. The surgical management of early-stage breast cancer is still based on the anatomic extent of the disease in the breast and axilla. However, it is hoped that by individualizing risk of locoregional recurrence and radiosensitivity with molecular subtyping/genomic classifiers, use and/or extent of adjuvant radiotherapy could be tailored. Prospective clinical trials are currently underway to test the validity of this approach.

The same group recently published data from a randomized trial of BCS with or without XRT.[20] They identified a low-risk subgroup of patients to be considered for XRT omission (age $>$ 60 years, T1 disease, luminal A, with 1.3% 10-year risk of LRR without XRT). The LUMINA trial described later was designed to validate these findings prospectively.

The aforementioned studies suggest that the LRR rate for the basal/triple-negative subtype is higher than that of the luminal A and B subtypes and similar to that of the HER2 subtype. However, 2 studies have shown no differences in the risk of LRR between triple-negative and non–triple-negative subtypes.[21,22] The results of these 2 studies are not necessarily contradictory to the ones presented previously because HER2-positive patients were included in the non–triple-negative cohort and all patients were treated in the pretrastuzumab era, which likely inflated the LRR risk for the non–triple-negative cohorts.

Additional support for the hypothesis that luminal tumors are more radiosensitive than triple-negative and HER2-positive tumors (in the pretrastuzumab era) is provided by Kyndi et al,[23] who evaluated tumor subtypes as predictors of LRR in 1,000 of the 3,083 patients with high-risk BC randomly assigned to postmastectomy radiation therapy (PMRT) in the Danish Breast Cancer Cooperative Group (DBCG) protocol 82b and 82c trials. In general, PMRT is defined as radiation to the chest wall and draining lymphatics. Median follow-up for patients who were alive was 17 years. Significantly smaller improvements in local control from PMRT were observed in the triple-negative (HR, 0.33; $P = .001$) and hormone receptor–negative/HER2-positive subtypes (HR, 0.53; $P = .2$) compared with the hormone receptor–positive/HER2-negative subtype (HR, 0.09; $P < .01$).

Furthermore, significantly improved overall survival after PMRT was seen only among patients characterized by good prognostic markers, including hormone receptor–positive/HER2-negative disease. These findings are in agreement with the observations from studies of molecular subtypes and LRR in patients treated with BCS plus XRT and indicate potentially higher sensitivity of the high-risk ER-positive tumors to XRT.

GENOMIC CLASSIFIERS FOR PREDICTION OF RISK OF LRR IN PATIENTS WITH INVASIVE BC

The demonstration of BC intrinsic subtypes based on microarray gene expression profiling[1,47] signaled the beginning of the genomic era in BC that has led to the development of several commercially (and noncommercially) available genomic classifiers developed to predict distant recurrence.[1,3-13] A logical next step was to evaluate these classifiers for prediction of LRR (Table 2).

Mamounas et al[32] evaluated the association between the 21-gene recurrence score (RS) in patients with node-negative, ER-positive BC from 2 National Surgical Adjuvant Breast and Bowel Project (NSABP) clinical trials (B-14 and B-20) in patients treated with no adjuvant therapy, tamoxifen, or tamoxifen plus chemotherapy. RS was a significant predictor of LRR in all 3 groups. In tamoxifen-treated patients, the 10-year risk of LRR was 4.3% with an RS of 0-17, 7.2% with an RS of 18-30, and 15.8% with an RS $\geq$ 31 ($P < .001$). This association provided biologic insights into LRR but had limited clinical implications regarding tailoring XRT use because, in the modern era, patients with high RS would receive chemotherapy. When the association between RS and risk of LRR was examined in patients treated with chemotherapy plus tamoxifen, the 10-year risk of LRR was 1.6% for RS of 0-17, 2.7% for RS of 18-30, and 7.8% for RS $\geq$ 31 ($P = .028$).

The low LRR risk in patients with low RS ($<$ 18) was recently confirmed in a large contemporary cohort of

TABLE 1. Studies Evaluating the Association Between Breast Cancer Subtypes and Locoregional Recurrence

Assessment of Receptor Subtypes	Patient Population	% of Patients Receiving Chemotherapy	% of Patients With Positive Nodes	Locoregional Recurrence						
				Lum A	Lum B	HER2	Luminal HER2	TNBC/Basal	Unclassified/ TNP Nonbasal	Study
IHC, FISH (HER2): 75% Lum A, ER+/PR+/HER−; 2%-10% Lum B, ER+/PR+/HER2+; 4% HER2, ER−/PR−/HER2+; 11% TNBC, ER−/PR−/HER2−	793 patients, BCS+XRT Dana-Farber/ Brigham Women's Hospital/ Massachusetts General Median FU, 70 months No trastuzumab 1998-2001	45.9	28.4	0.8% (5 years)	1.5% (5 years)	8.4% (5 years)	See Lum B	7.1% (5 years)	NA	Nguyen et al[16]
IHC, FISH (HER2) and tumor grade: 63% Lum A, ER+/PR+/HER2−, grade 1-2; 14% Lum A, ER+/PR+/HER2−, grade 3; 7% Lum B, ER+/PR+/HER2+; 4% HER2, ER−/PR−/HER2+; 12% TNBC, ER−/PR−/HER2−	1,434 patients, BCS+XRT Dana-Farber/ Brigham Women's Hospital/ Massachusetts General Median FU, 85 months No trastuzumab 1997-2006	46	26	0.8% (5 years)	2.3% (5 years)	10.8% (5 years)	1.1% (5 years)	6.7% (5 years)	NA	Arvold et al[17]
IHC and FISH (HER2): 79% Lum A, ER+/PR+/HER2−; 4.6% Lum B, ER+/PR+/HER2+; 2.6% HER2, ER−/PR−/HER2+; 10.4% basal, ER−/PR−/HER2−; CK5/6+ 3.2% unclassified, ER−/PR−/HER2−, CK5/6−	498 patients on trial of BCS+XRT with or without tumor bed boost St George Hospital Median FU, 84 months No trastuzumab 1997-2005	33	29	2% (5 years) 4.8% (10 years)	4.3% (5 years) 8.6% (10 years)	15.3% (5 years) 15.3% (10 years)	See Lum B	14.8% (5 years) 17.3% (10 years)	12.6% (5 years) 12.6% (10 years)	Millar et al[18]

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TABLE 1. Studies Evaluating the Association Between Breast Cancer Subtypes and Locoregional Recurrence (continued)

Assessment of Receptor Subtypes	Patient Population	% of Patients Receiving Chemotherapy	% of Patients With Positive Nodes	Locoregional Recurrence						Study
				Lum A	Lum B	HER2	Luminal HER2	TNBC/Basal	Unclassified/ TNP Nonbasal	
Tissue microarray: 43% Lum A, ER+/PR+, Ki-67 < 14%; 24% Lum B, ER+/PR+, Ki-67 ≥ 14%; 6% Lum HER2, ER+/PR+/ HER2+; 8% HER2, ER−/PR−/HER2+; 10% TNBC, ER−/PR−/ HER2−, EGFR+, CK5/ 6+; 9% TNP nonbasal, ER−/PR−/HER2−, EGFR−	2,985 patients British Columbia Registry BCS+XRT or MAST 1986-1992	0	43	BCS+XRT 8% (10 years) MAST 8% (10 years)	BCS+XRT 10% (10 years) MAST 14% (10 years)	BCS+XRT 21% (10 years) MAST 17% (10 years)	BCS+XRT 9% (10 years) MAST 20% (10 years)	BCS+XRT 14% (10 years) MAST 19% (10 years)	BCS+XRT 8% (10 years) MAST 13% (10 years)	Voduc et al[19]
IHC and FISH: 63% Lum A, ER+/PR+/HER2−; 10% Lum B, ER+/PR+/ HER2+; 12% HER2, ER−/PR−/ HER2+; 15% TNBC, ER−/PR−/ HER2−	1,000 patients on Danish Breast Cancer Cooperative Group trials of MAST with or without XRT 1982-1990	56	94	MAST+XRT 3% (15 years) MAST alone 32% (15 years)	MAST+XRT 3% (15 years) MAST alone 48% (15 years)	MAST+XRT 21% (15 years) MAST alone 33% (15 years)	See Lum B	MAST+XRT 15% (15 years) MAST alone 32% (15 years)	NA	Kyndi et al[23]
IHC and Herceptest (Agilent, Santa, Clara, CA; HER2): 76% non-TNBC, ER+, PR+, or HER2+; 24% TNBC, ER−/PR−/ HER2−	482 patients treated with BCS+XRT at Yale 1980-2003	47	25	17% (5 years; all non–triple negative together, including high-risk HER2+)				17% (5 years)	NA	Haffty et al[21]
IHC (Cb11 monoclonal antibody for HER2): 89% non-TNBC, ER+, PR+, or HER2+; 11% TNBC, ER−/PR−/ HER2−	1,601 patients treated at Henrietta Banting Breast Cancer 1987-1997	28	37	12%				13%	NA	Dent et al[22]

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TABLE 1. Studies Evaluating the Association Between Breast Cancer Subtypes and Locoregional Recurrence (continued)

				Locoregional Recurrence						
Assessment of Receptor Subtypes	**Patient Population**	**% of Patients Receiving Chemotherapy**	**% of Patients With Positive Nodes**	**Lum A**	**Lum B**	**HER2**	**Luminal HER2**	**TNBC/Basal**	**Unclassified/ TNP Nonbasal**	**Study**
Tissue microarray: 52% Lum A, ER+/PR+, Ki-67 < 14%; 33% Lum B, ER+/PR+, Ki-67 ≥ 14%; 4% Lum HER2, ER+/PR+/HER2+; 3% HER2, ER−/PR−/HER2+; 6% basal ER−/PR−/HER2−, EGFR+, CK5/6+; 1% TNP nonbasal, ER−/PR−/HER2−, EGFR−	501 patients on a trial of BCS *v* BCS+XRT 1992-2000	0	0	BCS+XRT 3.3% (10 years) BCS alone 7.3% (10 years)	BCS+XRT 7.8% (10 years) BCS alone 13.3% (10 years)	BCS+XRT (all high-risk subtypes grouped together) 6% (10 years) BCS alone 37.9% (10 years)				Liu et al[20]

Abbreviations: +, positive; −, negative; BCS, breast-conserving surgery; CK5/6 = cytokeratin 5/6; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FISH, fluorescence in situ hybridization; FU, follow-up; HER2, human epidermal growth factor receptor; IHC, immunohistochemistry; Lum A, luminal A; Lum B, luminal B; MAST, mastectomy; NA, not applicable; PR, progesterone receptor positive; TNBC, triple-negative breast cancer, TNP, triple-negative phenotype; XRT, radiation therapy.

TABLE 2. Studies Evaluating the Association Between Genomic Classifiers and Locoregional Recurrence

Assay	Description	Validation Sets	Clinical Trial Prospective Validation	References
Oncotype DX (Genomic Health, Redwood City, CA)	21-gene recurrence score originally designed to predict risk of distant metastases but also shown to be independently prognostic for LRR	Patients from NSABP B-14/B-21, n = 355 placebo, n = 895 tamoxifen, n = 424 tamoxifen and chemotherapy (1982-1993)	IDEA, MA.39	Mamounas et al[32]
		1,396 MSKCC early-stage ER-positive/HER2-negative patients (2008-2013)		Turashvili et al[33]
		316 patients from SWOG 8814 (1989-1995)		Woodward et al[35]
		1,065 patients from NSABP B-28 (1995-1998)		Mamounas et al[29]
		338 patients on ECOG E2197 (1998-2000)		Solin et al[28]
MammaPrint	70-gene signature designed to predict distant metastases but also shown to be prognostic for LRR	1,053 patients with invasive BC treated in Netherlands (1984-2006)		Drukker et al[27]
EndoPredict	8-gene signature designed to predict distant metastases but also found to be prognostic for LRR	1,324 patients on ABCSG-8 trial (1996-2004)		Fitzal et al[30]
PAM50 (Prosigna; NanoString, Seattle, WA)	58-gene risk of recurrence signature designed to predict risk of distant metastases; also shown to be prognostic for LRR	1,308 patients on ABCSG-8 trial (1996-2004)	PRECISION, EXPERT	Fitzal et al[34]
Wound response signature	70-gene signature known to predict metastasis-free and overall survival; also shown to be prognostic for LRR	295 patients with stage I/II BC treated at Netherlands Cancer Institute (1984-1995)		Nuyten et al[31]
Cheng et al[36] local recurrence score	34-gene signature designed to predict local recurrence risk	158 patients with invasive BC treated with mastectomy and no radiation at Duke or Koo Foundation Sun Yat-Sen Cancer Center (1990-2001)		Cheng et al[36]
DBCG-RT gene profile	7-gene signature designed to predict local recurrence risk	273 patients on the DBCG82b/c trials (1982-1990)		Tramm et al[37]
Radiation sensitivity score	51-gene score enriched for cell cycle arrest and DNA damage response aimed to assess radiation sensitivity; prognostic for local recurrence, predictive of radiation benefit	295 patients with stage I/II BC treated at Netherlands Cancer Institute (1984-1995)		Speers et al[38]
Radiosensitivity index	10-gene expression score developed to assess radiation sensitivity; prognostic for local recurrence, predictive of radiation benefit	343-patient data set from 4 Dutch centers and 1 French center (1984-2002)		Eschrich et al,[39] Torres-Roca et al[40]
Genomically adjusted radiation dose	Combination of radiosensitivity index and the linear quadratic model; prognostic for local control and predictive of response to radiation across a range of radiation doses	343-patient data set from 4 Dutch centers and 1 French Center (1984-2002), as well as 643 patients treated from Total Cancer Care Protocol of 17 institutions (2006-2018)		Ahmed et al[45]
Single-sample radiosensitivity gene expression predictor	248-gene expression panel designed for low-quality RNA samples on the NanoString platform; prognostic for local recurrence, predictive of radiation benefit	336 Swedish patients (1983-2009)		Sjöström et al[41]

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TABLE 2. Studies Evaluating the Association Between Genomic Classifiers and Locoregional Recurrence (continued)

Assay	Description	Validation Sets	Clinical Trial Prospective Validation	References
Immune gene signature	34 genes associated with radiation sensitivity and 119 genes associated with immune response; predictive of radiation benefit	1,439 patients from the Molecular Taxonomy of Breast Cancer International Consortium cohort		Cui et al[42]
Adjuvant Radiotherapy Intensification Classifier	27-gene expression score; prognostic for local recurrence and predictive of radiation benefit	748 patients from SweBCG91-RT (1991-1997)		Sjöström et al[46]

Abbreviations: BC, breast cancer; DBCG, Danish Breast Cancer Cooperative Group; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HER2, human epidermal growth factor receptor; LRR, locoregional recurrence; MSKCC, Memorial Sloan Kettering Cancer Center; NSABP, National Surgical Adjuvant Breast and Bowel Project.

node-negative, ER-positive/HER2-negative patients from the Memorial Sloan Kettering Cancer Center.[33] Most patients were treated with BCS plus XRT (66.6%) or total mastectomy alone (29.7%). Most patients (84.8%) received endocrine therapy alone, whereas 12.1% of patients were treated with chemotherapy plus endocrine therapy. With a median follow-up of 52 months, LRR rate was 0.9% overall and 0.7% in patients treated with adjuvant endocrine therapy only.

Similar findings were reported by Drukker et al,[27] who evaluated the 70-gene signature (MammaPrint; Agendia, Irvine, CA) in patients with T1-3, N0-1 disease. MammaPrint was significantly associated with LRR risk, with 10-year LRR rates of 6.1% for low-risk scores and 12.6% for high-risk scores. Adjusting the 70-gene signature in a competing risk model for clinicopathologic factors (age, tumor size, grade, hormone receptor status, LVSI, nodal stage, surgical treatment, endocrine treatment, and chemotherapy) resulted in a multivariable HR of 1.73 (95% CI, 1.02 to 2.93; $P = .042$).

Fitzal et al[30,34] evaluated both the PAM50 assay and the 8-gene assay EndoPredict (EP; Myriad Genetics, Salt Lake City, UT) in the ABCSG-8 trial of postmenopausal, ER-positive/HER2-negative patients treated with endocrine therapy. The majority of the patients (79%) were treated with BCS. With a median follow-up of 11 years, 10-year local recurrence–free survival (LRFS) risk was significantly associated with PAM50 risk of recurrence (ROR; 98.4% in the low/intermediate ROR group *v* 94.4% in the high ROR group). ROR score was the only significant independent predictor of LRR in multivariable analysis. For EP, the 10-year LRFS was significantly worse among patients with high-risk lesions (91%) versus those with low-risk lesions (97.5%; HR, 1.31; 95% CI, 1.16 to 1.48; $P < .005$). The groups that received BCS and mastectomy had similar LRR rates ($P = .879$). In a subgroup of BCS patients randomly assigned to breast XRT or no XRT,[30,48] EP was a significant predictor of LRR but not predictive of benefit from breast XRT. Breast XRT significantly improved LRFS both in the EP low-risk cohort (10-year LRR decreased from 11.1% to 0.2%; $P < .005$) and in the EP high-risk cohort (10-year LRR decreased from 12.0% to 2.5%; $P < .005$).

Noncommercially available predictors of LRR include a 34-gene expression profile developed using DNA microarray analysis by Cheng et al[36] that was significant for predicting LRR risk in patients subdivided by nodal stage. In addition, Nuyten et al[31] showed that a microarray-based 70-gene expression profile developed as a wound-response signature independently predicted BCS patients at high (29%) versus low (5%) risk of LRR at 10 years.

Currently, there are no commercially available genomic classifiers that identify subgroups of patients with BC who can be spared breast XRT after BCS. Several prospective trials (single-arm or randomized) are currently underway to address this question in patients with early-stage disease treated with BCS. The LUMINA trial (ClinicalTrials.gov identifier: NCT01791829) is enrolling patients ≥ 55 years old with T1N0 luminal A tumors in a single-arm prospective observation trial, with a primary end point to measure the 5-year ipsilateral breast recurrence rate. The IDEA trial (ClinicalTrials.gov identifier: NCT02400190) completed enrollment of postmenopausal patients with T1N0 ER-positive BC and low RS (< 18) onto a single-arm prospective observation trial, with a primary end point of 5-year LRR. The PRECISION trial (ClinicalTrials.gov identifier: NCT02653755) is enrolling women > 50 years old with T1N0 ER-positive BC and low PAM50 ROR score onto a single-arm prospective observation trial, also with a primary end point of 5-year LRR. The EXPERT trial (ClinicalTrials.gov identifier: NCT02889874) is a randomized study of BCS with or without XRT in T1N0 ER-positive BC in women older than 50 years with a PAM50 ROR of < 60. Until results from these trials become available, BCS plus XRT remains the standard of care for the majority of patients with invasive BC.

GENOMIC PROFILING AND RISK OF LRR IN PATIENTS WITH NODE-POSITIVE BC

The significant association between LRR and the 21-gene RS in node-negative patients treated with chemotherapy

and endocrine therapy provided rationale for evaluating RS in node-positive patients, hoping that improved LRR stratification could help to tailor PMRT and/or regional nodal XRT use. Three studies have reported such an association based on retrospective assessment of RS in exclusively node-positive patients included in randomized clinical trials of adjuvant chemotherapy plus endocrine therapy (NSABP B-28, Eastern Cooperative Oncology Group [ECOG] E2197, and SWOG 8814).[28,29,35]

In the NSABP B-28 trial, patients with positive nodes were randomly assigned to doxorubicin plus cyclophosphamide with or without paclitaxel. Patients ≥ 50 years old and those < 50 years old with ER-positive and/or PR-positive tumors also received tamoxifen. Per protocol, BCS patients received breast XRT, but mastectomy patients did not receive XRT. RS was obtained in 1,065 ER-positive patients.[29] With median follow-up of 11.2 years, the 10-year cumulative incidence of LRR was 3.3%, 7.2%, and 12.3% for patients with low RS (0-17), intermediate RS (18-30), and high RS (≥ 31), respectively ($P < .001$). In multivariable regression analysis, RS remained an independent predictor of LRR (HR, 2.61 for a 50-point difference in RS; $P = .008$), along with pathologic nodal status (HR, 1.91 for ≥ 4 *v* 1-3 positive nodes; $P = .007$) and tumor size (HR, 1.28 for a 1-cm difference; $P = .015$). These findings suggest that RS can be used along with clinicopathologic factors to better stratify risk of LRR in node-positive, ER-positive patients treated with chemotherapy plus endocrine therapy to better select appropriate candidates for PMRT and/or regional nodal irradiation after BCS and breast XRT.

Solin et al[28] evaluated RS for prediction of LRR risk in patients treated with BCS plus XRT with 1-3 positive nodes in the ECOG E2197 study comparing 2 chemotherapy regimens. In their study population, 10-year rates of local recurrence were 3.2%, 2.0%, and 10.1% for low, intermediate, and high RS, respectively, which was not statistically significant ($P = .17$). However, as a continuous variable, RS was a significant predictor of LRR (HR, 2.66; $P = .03$).

Woodward et al[35] recently reported on the retrospective assessment of RS in patients treated on SWOG 8814, comparing chemotherapy plus endocrine therapy versus endocrine therapy alone in node-positive, hormone receptor–positive BC. Estimated 10-year cumulative LRR rates were 9.7% and 16.5% for low and intermediate or high RS, respectively (log-rank $P = .018$). The RS remained significantly associated with LRR in an analysis of mastectomy patients alone (low RS, 7.7%; intermediate or high RS, 16.8%; $P = .025$). In a subset analysis of patients with a mastectomy and 1-3 involved nodes who did not receive XRT, patients with RS < 18 had a low LRR rate (1.5%), suggesting this may be a population for omission of PMRT.

These findings suggest that genomic profiling can significantly predict risk of LRR in node-positive patients, and this association could have clinical implications regarding tailoring regional nodal XRT or PMRT. However, before such an approach becomes accepted clinical practice, validation in a prospective clinical trial is needed. Such a trial is NCIC MA.39, which is currently accruing patients through the National Cancer Trials Network (ClinicalTrials.gov identifier: NCT03488693). MA.39 includes patients with ER-positive/HER2-negative, node-positive BC and a 21-gene RS of < 18. Mastectomy-treated patients are randomly assigned to chest wall plus regional nodal XRT versus no XRT, whereas patients treated with BCS are randomly assigned to breast plus regional nodal XRT versus breast XRT only. Patients are eligible if they have 1-3 positive nodes after axillary lymph node dissection, 1-2 positive nodes after BCS plus sentinel lymph node biopsy, or only 1 positive node after mastectomy plus sentinel lymph node biopsy. The primary objective of the trial is to determine whether omitting regional nodal XRT after BCS or PMRT in mastectomy patients is noninferior to its use in women with ER-positive BC, 1-3 positive axillary nodes, and an RS of < 18 treated with endocrine therapy. A total of 2,140 patients will be included in the study. Mastectomy-treated patients are randomly assigned to PMRT versus no PMRT, whereas BCS patients are randomly assigned to breast XRT with or without regional nodal XRT.

GENOMIC PROFILING FOR PREDICTION OF XRT BENEFIT

The aforementioned trials were designed to identify patient populations with a low risk of LRR, in whom the addition of chest or breast XRT or regional nodal XRT would not add substantial absolute benefit in local control. Another approach to tailor use of XRT is development of genomic classifiers predictive of XRT benefit, rather than LRR.

Tramm et al[37] attempted to develop a genomic predictor of XRT benefit in patients with high-risk BC treated with systemic therapy and randomly assigned to PMRT or no PMRT. Seven genes were identified, and the derived DBCG-RT profile divided the patients into high LRR risk and low LRR risk groups. PMRT significantly reduced risk of LRR in high LRR risk patients but not in low LRR risk patients.

Speers et al[38] developed a new genomic predictor for XRT sensitivity using clonogenic survival assays for BC cell lines exposed to XRT. Their 51-gene radiation sensitivity score (RSS) is enriched for genes involved in cell cycle arrest and DNA damage response. In a data set of patients undergoing BCS plus XRT, RSS was more strongly predictive of LRR than any clinicopathologic factors.

Eschrich et al[49] developed a generalized genetic assay for radiosensitivity by performing clonogenic survival studies in 48 different human cancer cell lines exposed to XRT. This radiosensitivity index (RSI) was validated in established databases of patients treated with BCS plus XRT or mastectomy without XRT.[39] BCS plus XRT patients predicted to

be radiosensitive by RSI had improved 5-year relapse-free survival (RFS) compared with radioresistant patients (95% *v* 75%, respectively; $P = .0212$). In patients treated with mastectomy alone, there was no difference in 5-year RFS between radiosensitive and radioresistant patients (71% *v* 77%, respectively; $P = .67$). When combining RSI with molecular subtype,[40] patients at greatest risk of LRR had triple-negative and radioresistant disease (HR, 0.37; $P = .02$).

Sjöström et al[41] used fresh frozen tissue from 336 patients undergoing BCS with or without XRT to develop a new radiosensitivity assay using the NanoString (Seattle, WA) platform suitable for low-quality RNA. In BCS patients with high risk of LRR, they identified 3 groups. In the low LRR risk/low-radiosensitivity group, XRT would not reduce risk of LRR. In the second group of patients, XRT was recommended as a result of high radiosensitivity. In the third group, escalated treatment was recommended, because their tumors had a high risk of LRR that was not significantly reduced by XRT.

More recently, Sjöström et al[46] identified a new 27-gene Adjuvant Radiotherapy Intensification Classifier (ARCTIC) using publicly available gene databases with known outcomes. This included a database of 336 patients with early-stage BC treated in Sweden with surgery first from 1983 to 2009,[41] a database of 295 patients with early-stage BC treated in the Netherlands from 1984 to 1995 treated with BCS or mastectomy,[2] and a database of 343 patients with early-stage BC treated with BCS treated in the Netherlands or France between 1984 and 2002.[50] It should be noted that these databases provided long-term follow-up; however, unlike the modern treatment regimens, no patients in these databases received neoadjuvant chemotherapy or trastuzumab as a part of their care.

ARCTIC scores were calculated for patients treated with BCS with or without XRT on the SweBCG91 trial. Paraffin-fixed tissue samples were available for 922 patients on the SweBCG91 trial, of which 748 had sufficient RNA for analysis.[46] Using these samples, they compared the results of ARCTIC to 8 different genomic signature scores from the aforementioned studies.[27,29,32,38,39,41,42,44] ARCTIC outperformed the other gene expression scores for predicting elevated risk of LRR as well as benefit from XRT. Patients with low ARCTIC score had a large XRT benefit with 10-year LRR risk reduced from 21% to 6% (HR, 0.33; $P < .001$). Patients with high ARCTIC score had a higher LRR risk and less XRT benefit, with 10-year LRR risk reduced from 32% to 25% (HR, 0.73; $P = .23$).

Several other groups have used publicly available BC gene data sets to develop and test radiosensitivity signatures. Cui et al[42] combined radiosensitivity genes with an immune signature, including genes involved in antigen processing and presentation. In the Molecular Taxonomy of Breast Cancer International Consortium cohort, patients predicted as XRT sensitive and immune effective had significantly improved survival with XRT (HR, 0.43; $P = .022$). In patients predicted as XRT resistant and immune defective, survival was worse with XRT (HR, 1.69; $P = .045$). Jang and Kim[43] used The Cancer Genome Atlas data set to develop and test a radiosensitivity signature. In radiosensitive patients who received XRT, there was improvement in recurrence-free survival (HR, 0.45; $P = .008$). The signature was not predictive of outcome in patients without XRT as a component of their care. Zhang et al[44] developed a centromere and kinetochore gene expression score (CES) signature that correlated with genomic instability. Patients with a high CES score treated with XRT had a significant improvement in overall survival (HR, 0.279; $P = .008$) and disease-free survival (HR, 0.254; $P = .016$). In patients with low CES score, there was no difference with XRT addition to the treatment course for overall survival (HR, 1.309; $P = .58$) or disease-free survival (HR, 0.95; $P = .98$).

The aforementioned studies identify gene expression assays that are predictive of XRT sensitivity but not correlated to XRT dose. Ahmed et al[45] integrated the RSI into the linear quadratic model used in radiobiology to provide the relationship between cell survival and dose. The resultant genomically adjusted XRT dose (GARD) score was calculated for patients with triple-negative BC in 2 independent data sets. Using the median GARD score as the cutoff between high and low score, GARD as a dichotomous variable was significant for LRR. In a model of GARD score versus XRT dose, optimal XRT was achieved in 78% of patients with a dose of 60 Gy. At 70 Gy, 91% of patients would receive optimal XRT.

DISCUSSION AND FUTURE DIRECTIONS

Although currently available evidence with molecular subtyping and genomic profiling provides great insight on tumor biology and its locoregional behavior, such evidence has not yet translated into meaningful changes in locoregional therapy approaches. In the setting of ER-positive/HER2-negative disease, multiple secondary analyses of randomized studies independently show that RS correlates to LRR similar to known prognostic variables, such as LVSI or stage. As such, RS may be incorporated into estimations of LRR risk. However, care must be taken when omitting XRT off protocol among women with low RS given the benefit from breast XRT in reducing in-breast recurrence after BCS and the benefit from PMRT and regional nodal XRT on disease-free survival.

Minimal data are available on the utility of genomic assays for LRR prognosis in the setting of neoadjuvant chemotherapy or with use of trastuzumab, and this remains a significant gap in knowledge. Development of predictive assays are promising but yet unproven. Studies are needed to prospectively correlate pathologic response to XRT with the existing genetic assays for radiosensitivity. Enrollment

on prospective studies such as MA.39 is strongly encouraged, and development of new trials to test the hypothesis that genomic assays can predict XRT benefit is greatly needed.

By individualizing risk of LRR and radiosensitivity with molecular subtyping/genomic classifiers, it is hoped that use and/or extent of adjuvant XRT could be tailored. Studies such as MA.39 will provide information on whether these assays can guide decision making for PMRT in patients with 1-3 lymph nodes. Use of LRR and radiosensitivity assays in patients undergoing BCS may help to identify patients at sufficiently low risk of LRR to omit breast XRT. Tailoring of XRT dose or adding radiosensitizers could be considered to improve outcome in patients with high LRR risk and radioresistant tumors. Another provocative, yet unaddressed, question is whether use of genomic assays could identify a population of early-stage, hormone receptor–positive patients with primarily local recurrence risk rather than distant recurrence risk, who could be adequately treated with surgery plus XRT alone, without the need for 5 years of adjuvant endocrine therapy.

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AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Molecular Predictive and Prognostic Markers in Locoregional Management

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