The clinical utility of gene expression assays in breast cancer patients with 0–3 involved lymph nodes

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***Abstract*:** Multigene expression assays are prognostic for recurrence in hormone-receptor positive 2 (HER-2) negative breast cancer, and, in some cases, predictive of benefit from chemotherapy or extended endocrine therapy. The results of these assays may be used to guide treatment recommendations for early HER-2 negative breast cancer. We review the results of trials establishing the clinical utility of several commercially available gene expression assays.

Keywords: breast cancer, chemotherapy, gene expression profiling, hormone receptor-positive breast cancer, hormone therapy

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Introduction

Breast cancer (BCA) mortality has declined substantially over the last 40 years, in part due to the use of adjuvant systemic therapies to decrease the risk of recurrence, including adjuvant chemotherapy, endocrine therapy, and targeted anti- human epidermal growth factor receptor 2 (HER2) therapy. For adjuvant endocrine therapy and anti-HER2 therapy, predictive factors for therapeutic benefit include hormone receptor expression and HER2/neu overexpression, respectively. In contrast, adjuvant chemotherapy recommendations have traditionally been based on clinicopathological factors associated with higher distant recurrence risk, including larger tumor size, higher tumor grade, and axillary nodal involvement. More recently, multigene expression assays have been shown to not only be prognostic for distant recurrence in estrogen-receptor positive, HER2-negative breast cancer, but also predictive of chemotherapy benefit. The integration of tumor genomic information with standard clinicopathologic risk factors provides additional personalization and refinement of prognostic estimates; it also allows for more precise estimation of absolute chemotherapy benefit.

The three essential elements of any diagnostic test include its analytic validity (ie, reproducibility and accuracy), clinical validity (accuracy of identifying presence, absence, or risk of a specific disease or outcome), and clinical utility. Clinical utility is defined as a test result that may prevent or ameliorate adverse health outcomes such as mortality, morbidity, or disability through the adoption of efficacious treatments; in other words, the test result may effect a treatment decision regarding adjuvant chemotherapy use, and patients benefit from that guidance by providing a greater level of assurance of benefit from the treatment. Although all tests that have clinical utility also exhibit clinical validity, most tests with clinical validity do not also have clinical utility. Diagnostic tests that provide predictive information for benefit from specific therapies in addition to prognostic information for recurrence risk are inherently more likely to demonstrate clinical utility. This review will summarize the clinical utility of several commonly used multiparameter gene expression assays (Table 1), including the Oncotype DX Recurrence Score (RS) (Genomic Health, Exact Sciences, Madison WI), MammaPrint (Agendia, Inc., Irvine, CA), PAM50 Risk of Recurrence (ROR) score (Prosigna, Veracyte, San Francisco, CA), EndoPredict (EP) (Myriad Genetics, Salt Lake City, UT), and Breast Cancer Index (BCI) (Biotheranostics, Inc., San Diego, CA), in early hormone receptor-positive (HR+) HER2

Table 1. Commercially available multiparameter gene expression assays.

Assay	Clinical factors incorporated	Year available*	Prognostic	Predictive
21 gene (Oncotype DX)	None**	2004	Yes	Chemotherapy benefit
70 gene (MammaPrint)	None	2007	Yes	Not determined
50 gene (Prosigna, PAM50)	Tumor size	2013	Yes	Not determined
12 gene (EndoPredict)	Tumor size, nodes	2014	Yes	Not determined
7 gene (Breast Cancer Index)	None***	2014	Yes	H/I predictive of EET benefit

*Year that assay became commercially available in the United States
**Tumor size, grade, and age incorporated into RSclin educational tool for N0 patients
***Tumor size and grade incorporated into prognostic model for N1 patients
EET, extended endocrine therapy; H/I, HOXB13/IL17BR expression ratio.

negative BCA. The evidence supporting the use of each assay is summarized in Table 2.

MammaPrint

A 70-gene prognostic classifier was first described using microarray analysis in a cohort of 117 patients under age 55 with early BCA.[36] The clinical validity of the 70-gene signature was subsequently validated using microarray analysis of archival frozen BCA tissue from 2 independent cohorts, including 295 patients at a single center,[1] and 307 patients treated at 5 centers,[2] which confirmed the higher distant recurrence risk in patients with a high-risk genomic signature, and demonstrated that genomic risk was a better predictor of prognosis than traditional clinicopathologic features or the Adjuvant! Online software.[2] The assay was subsequently analytically validated for use in formalin-fixed, paraffin embedded tissues; it is now commercially available as the MammaPrint assay.[37,38]

The 70-gene classifier using frozen tissue for analysis was prospectively evaluated in the MINDACT (Microarray In Node-Negative and 1-3 node-positive Disease may Avoid Chemo Therapy) trial, which enrolled 6693 women with BCA and up to 3 involved axillary nodes (N1), of whom 81% had ER-positive, HER2-negative disease. The assay has no role in the management of triple-negative breast cancer, given that 97% had a high-genomic risk, nor in HER2/neu positive breast cancer, where anti-HER2 therapy is indicated. The patients' genomic risk was assessed by MammaPrint testing and the clinical risk by a modified version of Adjuvant! Online.[3] Clinical low-risk was defined as axillary node-negative tumors up to 1 cm in size and high-grade, 2 cm in size and intermediate-grade, or 3 cm in size and low grade; in contrast, those not meeting these criteria were defined as high-risk (which also included any patients with positive axillary nodes). Patients with concordant high clinical and genomic risk received adjuvant chemotherapy, while patients with concordant low clinical and genomic risk did not. Patients with discordant risk were randomized to management based on clinical or genomic risk. As a result, patients who were clinically high-risk and genomically low-risk received chemotherapy if they were randomized to treatment by clinical risk and did not receive chemotherapy if they were randomized to treatment by genomic risk. In contrast, patients who were clinically low-risk and genomically high-risk received chemotherapy if randomized to treatment by genomic risk and did not receive chemotherapy if randomized to treatment by clinical risk. The trial's primary aim was to determine whether the subgroup of 644 patients with clinical high-risk but genomic low-risk randomized to no chemotherapy had a 5-year distant metastasis-free survival (DMFS) with a lower 95% confidence interval (CI) boundary of at least 92%. DMFS for this group was 94.7% (95% CI 92.5–96.2%), which met the study's primary endpoint.[3,4] The evaluation of the low clinical risk patients enrolled in the MINDACT trial demonstrated an excellent 8-year DMFS [94.7% (95% CI 93.8–95.6%)] for concordant clinical and genomic low-risk patients and showed a 3.6% DMFS decrement associated with high genomic

Table 2. Selected clinical trials evaluating gene expression assays.

Trial/author	Study design	Population	*N*	Adjuvant therapy	Findings
MammaPrint					
Van de vijver[1]	Prospective-retrospective	HR+, HR–, N0, N+, Stage I–II, age < 53	295	Varied	Prognostic for DR
TRANSBIG/Buyse[2]	Prospective-retrospective	HR+, HR–, N0, age < 61	307	None	Prognostic for DR and OS
MINDACT/Cardoso[3,4], van't Veer *et al.*[5], Piccart *et al.*[6]	Prospective randomized	HR+, HR–, HER2+, HER2–, N0, N1	6693	Randomization to treatment based on clinical risk *versus* genomic risk for patients with discordant clinical and genomic risks	Patients with clinical high and genomic low risk may be spared chemotherapy. Patients with concordant clinical and genomic low risk have excellent DMFS. Small chemotherapy benefit in women age < 50 with high clinical risk and low genomic risk.
Oncotype DX					
NSABP B14/Paik *et al.*[7]	Prospective-retrospective	HR+, N0	668	Tamoxifen	Prognostic for DR and OS
TransATAC/Dowsett *et al.*[8]	Prospective-retrospective	HR+, N0, N+, postmenopausal	1231	Tamoxifen, Anastrozole	Prognostic for DR
E2197/Goldstein *et al.*[9]	Prospective-retrospective	HR+, N0, N1	465	chemotherapy, ET	Prognostic for recurrence
NSABP B20/Paik *et al.*[10], Geyer *et al.*[11]	Prospective-retrospective	HR+, N0	651 (569 HER2-neg by RT-PCR)	Tamoxifen +/–chemotherapy (CMF/MF)	Predictive for chemotherapy benefit (10-year DR rate) for RS ⩾ 31 in overall population, and for RS > 25 in HER2-negative population
S8814/Albain *et al.*[12]	Prospective-retrospective	HR+, N+, postmenopausal	367	Tamoxifen +/–chemotherapy (CAF)	Predictive for chemotherapy benefit (DFS, OS, BCSS) for RS ⩾ 31
PlanB/Gluz *et al.*[13], Nitz *et al.*[14]	Prospective, non-randomized	HR+, HR–, HER2–, high-risk N0, N+	3198	Chemotherapy omitted for patients with HR+, N0-1, and RS ⩽ 11 ($n = 348$). All others randomized to one of 2 different chemotherapy regimens	Patients with HR+ N0-1 disease and RS ⩽ 11 have 5-year DFS of 94% without adjuvant chemotherapy
TAILORx/Sparano *et al.*[15–17]	Prospective randomized	HR+, HER2–, T1-2, N0	10,273	ET for RS 0-10; CET for RS ⩾ 26; randomization to CET *versus* ET for RS 11–25	RS 11-25: ET noninferior to CET; small chemotherapy benefit in women ⩽ 50 with RS 21–25 or high clinical risk and RS 16-20 RS 0-10: low DR with ET alone RS ⩾ 26: better outcomes with CET than expected if ET alone used

(continued)

Table 2. (continued)

Trial/author	Study design	Population	*N*	Adjuvant therapy	Findings
RxPONDER/Kalinsk *et al.*[18]	Prospective randomized	HR+, HER2–, N1, RS ⩽ 25	5083	Randomization to CET *versus* ET	No benefit to CET over ET in postmenopausal women; 5.2% improvement in iDFS with CET in premenopausal women, regardless of RS
ADAPT/Kuemmel *et al.*[19], Harbeck *et al.*[20]	Prospective, non-randomized	HR+, HER2–, N0, N+	5625	Neoadjuvant chemotherapy for high-risk disease (including RS > 25); adjuvant ET for low-risk (N0-1, RS 0-11) or intermediate-risk endocrine sensitive (N0-1, RS 12-25, Ki67 ⩽ 10% after a 3-week course of neoadjuvant ET)	RS > 25 can be used to select patients for neoadjuvant chemotherapy; iDFS in endocrine-sensitive patients with RS 12-25 is non-inferior to that of patients with RS ⩽ 11
Breast Cancer Index (BCI)					
Stockholm study, multi-institution cohort/Zhang *et al.*[21]	Prospective-retrospective	HR+, HER2–, N0	958	Tamoxifen, placebo	Prognostic for early (⩽5 years) and late (>5 years) DR
TransATAC/Sgroi *et al.*[22]	Prospective-retrospective	HR+, N0, postmenopausal	665	Tamoxifen, anastrozole	Prognostic for early (⩽5 years) and late (>5 years) DR
MA.17/Sgroi *et al.*[23]	Prospective-retrospective	HR+, N0, postmenopausal	249	Tamoxifen -> letrozole *versus* placebo	BCI (H/I)-high predictive for EET benefit
IDEAL/Noordhoek *et al.*[24], Liefers *et al.*[25]	Prospective-retrospective	HR+ HER2–, N0, N+, postmenopausal	908	ET × 5 yrs -> letrozole 2.5 *versus* 5 yrs	BCI (H/I)-high predictive for benefit from extended letrozole in both clinical high and low risk patients, and in patients at high genomic risk of recurrence
Trans-aTTom/Bartlett *et al.*[26], Sgroi *et al.*[27]	Prospective-retrospective	HR+, N+	583 and 789	Tamoxifen	BCI (H/I)-high predictive for EET benefit with tamoxifen; ER, PR, AR, and Ki67 not predictive for EET benefit
Prosigna (ROR)					
ABCSG8/Gnant *et al.*[28]	Prospective-retrospective	HR+, N0, N+, postmenopausal	1478	Tamoxifen, tamoxifen -> anastrozole	Prognostic for DR
TransATAC/Dowsett *et al.*[29]	Prospective-retrospective	HR+, HER–, N0, N+, postmenopausal	1017	Tamoxifen, anastrozole	Prognostic for DR
OPTIMA[30]	Prospective randomized	HR+, HER2–, N+, high-risk N0, age > 40	4500*	CET *versus* treatment assignment by ROR	In process

(continued)

Table 2. (continued)

Trial/author	Study design	Population	*N*	Adjuvant therapy	Findings
EndoPredict (EP and EPclin)					
ABCSG6,8/Filipits *et al.*[31], Dubsky *et al.*[32]	Prospective-retrospective	HR+, HER2–, N0, N+, postmenopausal	1702	Tamoxifen, tamoxifen -> anastrozole	EP and EPclin prognostic for early and late DR
GEICAM9906/Martin *et al.*[33]	Prospective-retrospective	HR+, HER2–, N+	555	CET	EP prognostic for MFS and OS
ABCSG6,8, TransATAC, GEICAM 2003/02, GEICAM9906/Sestak *et al.*[34]	Prospective-retrospective	HR+, HER2–, N0, N+	3746	ET, CET	EPclin prognostic for DR; indirect comparison suggests EPclin may be predictive for chemotherapy benefit.
Ettl *et al.*[35]	Prospective non-randomized	HR+, HER2–, N0, N1	373	ET, CET	EPclin prognostic for DMFS; EPclin high risk patients who received CET had trend to improved DFS *versus* high-risk patients who declined CET.

*Planned accrual.
AR, androgen receptor; CET, chemoendocrine therapy; DFS, disease-free survival; DMFS, distant metastasis-free survival; DR, distant recurrence; EET, extended endocrine therapy; ER, estrogen receptor; ET, endocrine therapy; H/I, HOXB13/IL17BR expression ratio; HR+, hormone receptor positive; HR–, hormone receptor negative; iDFS, invasive disease-free survival; MFS, metastasis-free survival; N+, node positive; N0, node negative; N1, 1-3 involved nodes; PR, progesterone receptor; T1, tumor size ⩽2 cm; T2, tumor size 2–5 cm.

risk. Although MINDACT was underpowered to determine chemotherapy benefit, clinical low-risk/genomic high-risk patients who received chemotherapy had a 1.5% [standard error (SE) ± 2.3] improvement in DMFS, which, in an updated analysis, was subsequently shown to be limited to patients aged 50 or under (5.4% improvement in DMFS).[5]

Oncotype DX 21-gene recurrence score

The clinical validity of Oncotype DX 21-gene recurrence score (RS) was established using retrospective analysis of archival tissue derived from prospective randomized trials. These studies demonstrated that RS was prognostic for recurrence in patients with node-negative (N0) and node-positive (N+) HR+ BCA treated with tamoxifen,[7] anastrozole,[8] and chemotherapy,[9] including when adjusted for clinicopathologic factors. Similar prospective-retrospective studies evaluating the RS in tumor specimens from patients enrolled in randomized trials comparing chemoendocrine therapy (CET) to endocrine therapy (ET) alone, demonstrated that high RS was predictive of chemotherapy benefit in N0 HR+ BCA,[10] and also in postmenopausal women with positive axillary nodes.[12]

Prospective trials evaluating the 21-gene RS

The omission of chemotherapy based on RS was prospectively evaluated as a part of the German PlanB trial. Originally designed as a randomized trial of two different chemotherapy regimens in N+ and high-risk N0 HER2 negative BCA, the trial was amended such that women with HR+ BCA, 0-3 involved axillary nodes, and RS ⩽ 11 received ET alone. These patients ($n = 348$) had excellent outcomes, with 5-year DFS = 94%.[13,14] While the number of enrolled women was small, this trial was the first to prospectively demonstrate that chemotherapy might be omitted in women with high-risk clinical features and low RS without compromising disease-free survival.

In order to prospectively evaluate the 21-gene assay's clinical utility in N0 BCA, the Trial Assigning IndividuaLized Options for Treatment (TAILORx) enrolled over 10,000 women with N0 ER+ BCA, who were assigned or randomized to treatment based on RS. Women with low RS (0–10) were assigned to ET alone, women with high RS (26–100) were assigned to CET, and women with a mid-range RS (11–25) were randomized to ET *versus* CET. The TAILORx trial adjusted the RS ranges from those originally defined in the 21-gene assay validation studies, in order to account for the absence of HER2-positive disease (which is invariably associated with high RS and benefit from anti-HER2 therapy plus chemotherapy), reflect the manner in which RS is used in clinical practice, and minimize potential for chemotherapy undertreatment in the randomized arms.[39]

The TAILORx trial demonstrated that ET alone was non-inferior to CET in N0 BCA with RS 11–25 [hazard ratio (HR) for invasive disease-free survival (iDFS) 1.08; 95% CI 0.94–1.24; $p = 0.26$], the primary trial endpoint, and freedom from recurrence of BCA at a distant site (HR 1.10, 95% CI 0.85, 1.41, $p = 0.48$), a secondary trial endpoint. Nine-year rates in the ET and CET groups were 83.3% *versus* 84.3% for IDFS, 94.5% and 95.0% for freedom from recurrence of BCA at a distant site, and 93.9% and 93.8% for overall survival (OS), respectively.[15] Subgroup analysis demonstrated an interaction between chemotherapy administration, age (or menopausal status), and RS, in which women under 50 (or premenopausal) with RS 16–25 received a small benefit from chemotherapy.[15] Subsequent analysis showed that this chemotherapy benefit was predominantly limited to premenopausal women age 45–50, suggesting that much of it was due to induction of premature menopause, and that intensification of endocrine therapy with ovarian suppression and an aromatase inhibitor might achieve similar results.[16] Patients enrolled in TAILORx with RS 0-10, who received ET alone, had a rate of distant recurrence of 1% at 5 years and approximately 3% at 9 years, confirming the excellent outcomes for those with a low RS in this range seen in the PlanB trial.[15,40] In contrast, women with RS ⩾ 26 treated with CET had a 5-year rate of freedom from distant recurrence of 93% (SE, 0.8%), with no differences evident based on chemotherapy regimen administered. The expected rate of distant recurrence without chemotherapy for these patients was estimated based on the chemotherapy treatment effect observed in the HER2-negative cohort of the NSABP B20 trial (which had demonstrated prediction of chemotherapy benefit with a RS of 26–100); the expected 5-year rate of freedom from distant recurrence in the TAILORx cohort was projected to be 78.8% (SE, 14.0%) without chemotherapy, suggesting a large absolute chemotherapy benefit for those with a high RS.[17]

The 21-gene assay's role in predicting chemotherapy benefit in women with N1 disease was prospectively evaluated in the Rx for Positive Node, Endocrine Responsive Breast Cancer (RxPONDER) trial. In this trial, 5083 women with HR+, HER2-negative BCA, with N1 disease and a 21-gene RS of 0–25 were randomized to CET *versus* ET. Postmenopausal women did not benefit from chemotherapy, with a 5-year iDFS of 91.9% for CET *versus* 91.6% for ET alone (HR = 0.97, $p = 0.82$), and 5-year OS of 96.2% *versus* 96.1% (HR = 0.96, $p = 0.79$). In contrast, premenopausal women had a 5.2% improvement in iDFS with chemotherapy [94.2% for CET *versus* 89.0%. for ET alone (HR = 0.54, $p = 0.0004$], which was similar in women with RS 0-13 and 14–25. OS was also improved in premenopausal women receiving CET (98.6% *versus* 97.3%, HR = 0.47, $p = 0.032$),[18] suggesting that the chemotherapy benefit observed solely in premenopausal women may have been largely due to a castration effect. The results of RxPONDER are therefore consistent with the findings of TAILORx and MINDACT and expand the population of women with HR+ BCA who can safely forgo chemotherapy.

Integration of clinicopathologic and genomic risk

A secondary analysis of TAILORx demonstrated that clinicopathologic factors (tumor size and grade) provide prognostic information for distant recurrence, but not predictive information for chemotherapy benefit.[16] No chemotherapy benefit was found in women 50 or younger with RS 16–20 and low clinical risk, who had a very low 9-year distant recurrence risk with ET alone (4.6% ± SE 1.5). Distant recurrence rates were substantially higher for those age ⩽ 50 with RS 16–20 and high clinical risk treated with ET alone (11.9% ± SE 3.9), and for ET-treated women ⩽ 50 with RS of 21–25, with both low (11.4% ± SE3.9) and high clinical risk (18.8% ± SE5.5). This substantially higher underlying distant recurrence risk contributed to the chemotherapy benefit observed in patients with RS of 16–20 and high clinical risk (6.5% ± SE 4.9%), and in those with RS 21–25 and low clinical risk (6.4 ± SE 4.9%) or high clinical risk (8.7 ± SE 6.2%).[16] Consistent with the TAILORx findings, a *post hoc* analysis of the MINDACT study also showed evidence of an absolute chemotherapy benefit of approximately 5% at 8 years in women under age 50 with high clinical risk and low genomic risk, indicating that the 70-gene assay was prognostic but not predictive of chemotherapy benefit.[6]

Online algorithms have been developed that formally integrate RS with clinical risk in N0 BCA. The RS-pathology-clinical (RSPC) algorithm, which incorporates patient age, tumor size and grade, and choice of endocrine therapy with RS, utilized data from patients enrolled in the NSABP B14 and TransATAC trials. The RSPC was shown to provide additional prognostic information regarding recurrence risk for patients with N0 disease compared with RS alone but did not predict chemotherapy benefit.[41] The new RSClin online tool was developed using patient-specific meta-analysis derived data from 10,004 patients in the TAILORx, B14, and B20 trials, and subsequently validated using data from 1098 patients in the Israeli Clalit health service registry. RSClin integrates prognostic information provided by age, tumor size, and grade with prognostic and predictive information provided by the 21-gene RS and provides estimates of 10-year risk of distant recurrence and absolute chemotherapy benefit. The RSClin algorithm was derived from a larger, more contemporaneously treated patient cohort than that used for RSPC and provides more prognostic information than either the RS or clinicopathologic features alone.[42] RSClin is currently available online for clinician use as an educational tool for shared decision making.

Patient management utilizing the combination of RS, clinicopathologic factors, and a dynamic Ki67 response to endocrine therapy was prospectively evaluated in both N0 and N+ patients in the Adjuvant Dynamic Marker-Adjusted Personalized Therapy (ADAPT) trial. The trial enrolled 5625 women with HR+ HER2-negative breast cancer. Patients were initially treated with 3 weeks of neoadjuvant ET, followed by a biopsy to evaluate post-treatment Ki67. During this time, RS was evaluated in patients with N0 and N1 disease. High-risk patients (defined as those with clinical N2 or N3 disease, RS > 25, Grade 3 disease >1 cm with a baseline Ki67 > 40%, and RS 12–25 without suppression of Ki67 to <10% on ET) were randomized to one of two neoadjuvant chemotherapy regimens (4 cycles of paclitaxel administered every 2 weeks *versus* 8 weeks of nab-paclitaxel, both followed by 4 cycles of dose dense epirubicin and cyclophosphamide), with a primary endpoint of pathologic complete response (pCR). The remaining patients, including those with a RS 0-10 or a RS 11–25 and post-ET

Ki67 ⩽ 10% received surgery followed by adjuvant ET, with a primary endpoint of iDFS. The results of the neoadjuvant portion of ADAPT showed that patients with RS > 25 were significantly more likely to achieve pCR than patients with lower RS of 11–25 and elevated Ki67 after ET (16.1% *versus* 7.2%; $p = 0.006$). In multivariable analysis, only RS and tumor size predicted for pCR, demonstrating that RS can be used to select HR+ HER2 negative BCA patients for neoadjuvant chemotherapy.[19] The adjuvant endocrine portion of ADAPT demonstrated noninferior 5-year iDFS for ET-responsive patients with RS of 12–25 and Ki67 ⩽ 10% after ET compared with patients with RS ⩽ 11 [92.6% (95% CI 90.8%, 94.0%) *versus* 93.9% (95% CI 91.8, 95.4%), respectively], which met prespecified criteria for noninferiority, thereby meeting the primary trial endpoint. 5-year distant DFS [95.6% (95% CI 94.2%, 96.7%) *versus* 96.3% (95% CI 94.6%, 97.5%) $p = 0.247$, respectively] and 5-year OS [97.3% (95% CI 96.1%, 98.1%) *versus* 98.0% (95% CI 96.7%, 98.9%), respectively] were also similar. Subgroup analysis demonstrated poorer outcomes for patients with RS 12–25 and 3 involved lymph nodes, who had 5-year distant DFS of 75.9% and therefore may be suboptimal candidates for management with ET alone.[20]

Breast Cancer Index

The breast cancer index (BCI) is composed of two assays: the ratio of expression of the homeobox protein Hox-B13 gene to that of the interleukin-17 receptor B gene (H/I) and the Molecular Grade Index (MGI), which evaluates the expression of five genes involved in tumor proliferation.[43] The BCI is prognostic for both early and late recurrence, with the MGI more sensitive to early recurrence and the H/I more sensitive to late recurrence.[21,22] Integration of tumor size and grade into the BCI algorithm was shown to add additional prognostic information for distant recurrence in patients with N1 disease.[44] In addition, the H/I component has been shown to predict for benefit from prolongation of adjuvant endocrine therapy, such that patients with high H/I derive benefit from extended endocrine therapy (EET) beyond five years, while patients with low H/I do not.[23]

The ability of BCI (H/I) score to predict the benefit of extension of ET with an aromatase inhibitor was examined in a prospective–retrospective evaluation of tissue samples from 908 women enrolled in the IDEAL (The Investigation on the Duration of Extended Adjuvant Letrozole) trial, which randomized postmenopausal women with early HR+ BCA who had completed 5 years of adjuvant ET to additional therapy with letrozole for either 2.5 *versus* 5 years.[45] Patients whose tumors were BCI (H/I)-high and who received 5 years of letrozole had an absolute benefit of 9.8% ($p = 0.011$). In contrast, additional letrozole did not decrease recurrence risk in patients whose tumors were BCI (H/I) low ($p = 0.835$). BCI (H/I) predicted for EET benefit in both patients who were at high clinical risk of recurrence (large tumors and/or N+ disease) ($p = 0.035$) and in patients with low clinical risk tumors ($p = 0.013$).[24] In addition, evaluation of these findings in the context of genomic risk, as calculated by the BCI prognostic score (which combines the MGI and H/I), showed that patients at high genomic risk for recurrence could be subdivided into a BCI (H/I)-high group, who had improved recurrence-free interval (RFI) with EET ($p = 0.020$), and a BCI (H/I)-low group, who did not ($p = 0.880$).[25]

The ability of BCI (H/I) to predict benefit from EET with tamoxifen was evaluated in the prospective-retrospective Trans-aTTom study, utilizing tumor tissue from 583 women with HR+ N+ BCA enrolled on the aTTom trial, which had randomized women who had completed 5 years of tamoxifen to an additional 5 years of treatment (10 years total) *versus* observation (5 years total treatment). Patients with BCI (H/I)-high disease had an 10.2% absolute reduction in risk of late recurrence with 10 years of tamoxifen ($p = 0.027$). In contrast, patients with BCI (H/I)-low disease did not derive benefit from additional tamoxifen (−0.2% RFI; HR = 1.07; 95% CI 0.69–1.65; $p = 0.768$).[26] ER, PR, AR, and Ki67 protein expression levels did not predict benefit from tamoxifen EET, and correlated weakly or not at all with BCI (H/I).[27] These studies demonstrate that BCI (H/I) has utility in selecting patients with HR+ BCA for EET.

Prosigna PAM50 Risk of Recurrence

The risk of recurrence (ROR) score incorporates the results of the PAM50 assay, which evaluates the expression of 50 cancer-related genes to classify tumors into intrinsic subtypes, with tumor size and node involvement, to generate the ROR score. The ROR score was evaluated in prospective-retrospective evaluations of tumor tissue from patients enrolled in the ABCSG-8[28] and

ATAC[29] trials, demonstrating that the ROR score assay was prognostic for recurrence. The ABSCG-8 prospective-retrospective trial evaluated 1478 tumor specimens from postmenopausal women with N0 and node-positive HR-positive BCA treated with endocrine therapy alone. Patients with low ROR had a 10-year distant recurrence-free survival (DRFS) of 96.7% (95% CI 94.6–98.0%), compared with 91.3% (95% CI 88.1–93.8%) for those with intermediate ROR and 79.9% (95% CI 75.7–83.4%) for those with high ROR.[28] The Prosigna score cutpoints for low- risk disease in the N0 setting are ⩽40 for low risk, 41–60 for intermediate risk, and 61–100 for high risk, corresponding to 10-year DR risks of 3%, 10%, and 16% respectively. In the node-positive setting, the cutpoints are ⩽40 for low risk and 41–100 for high risk, without an intermediate risk group, corresponding to 10-year DR of 6% and 24%, respectively.[46,47] The Prosigna assay is currently being evaluated prospectively in the ongoing Optimal Personalised Treatment of early breast cancer using Multi-parameter Analysis (OPTIMA) trial, in which women and men age 40 and above with HR+ HER2-negative BCA and up to 9 involved axillary nodes are randomized to adjuvant CET *versus* adjuvant therapy directed by the results of the Prosigna ROR assay.[30] Patients randomized to therapy directed by the Prosigna assay receive CET for ROR ⩾ 60, and patients receiving CET are blinded as to their randomization arm.[30]

EndoPredict

The EndoPredict (EPclin) risk score is generated by combining the results of the EndoPredict 12-gene expression assay with tumor size and node involvement. EPclin was validated in prospective-retrospective evaluations of tumor tissue from HR+ HER2-negative patients treated on one of 5 large clinical trials, which demonstrated that EPclin was prognostic for early and late recurrence in patients receiving ET alone[31,32,34] and CET.[33,34] EPclin was prospectively evaluated in 373 consecutive patients with ER+, HER2-negative, N0 or N1 BCA. Patients with high risk EPclin score (defined as >3.3 and corresponding to an estimated 10-year risk of DR of >10%) had a significantly poorer 3-years DMFS than patients with low-risk score (HR 5.18; 95% CI 1.04–25.74; $p = 0.0443$). The majority of high-risk patients were recommended to receive adjuvant chemotherapy, and 72% complied. These patients had a trend to improved 3-year DFS compared with high-risk patients who declined recommended chemotherapy (96.3% *versus* 91.5%; HR 0.32; 95% CI 0.10–1.05; $p = 0.061$).[35]

Incorporation of genomic assays into clinical practice guidelines

Gene expression assays have been incorporated into national and international clinical practice guidelines for the management of early HR+ HER2-negative BCA (summarized in Table 3). The National Comprehensive Cancer Network (NCCN) guidelines were updated in early 2021 to incorporate the results of the RxPONDER trial.[48]

The most recent American Society of Clinical Oncology (ASCO) guidelines for use of biomarkers to guide adjuvant BCA therapy was published in 2016,[49] and underwent focused updates in 2017, after publication of the MINDACT trial,[50] and in 2019, after publication of TAILORx.[51] These guidelines state that clinicians may use the Oncotype DX, MammaPrint, EndoPredict, PAM50 ROR, or BCI assays to guide therapy recommendations for patients with HR+, HER2-negative N0 BCA. The Oncotype DX, MammaPrint, and PAM50 assays received strong recommendations based on high quality evidence, while EndoPredict and BCI received moderate level recommendations based on intermediate quality evidence. MammaPrint was recommended only in patients at high clinical risk. In the node-positive setting, MammaPrint was recommended in patients at high clinical risk, but with the caveat that patients be informed that chemotherapy benefit could not be excluded.[49–51]

The European Society for Medical Oncology (ESMO) guidelines for breast cancer treatment, also published in 2019, state that gene expression assays may be used to determine recurrence risk when indications for adjuvant chemotherapy are unclear.[52] As a result, gene expression assays were not recommended in patients at very high (4 or more involved axillary nodes) or very low (small, well differentiated, strongly ER+, N0 tumors or tumors of favorable histologies) clinical risk of recurrence, or in patients with comorbidities that preclude chemotherapy. First generation assays (Oncotype and MammaPrint) received Grade A recommendations (strong evidence for efficacy, substantial clinical benefit, strongly recommended), while second generation assays (EndoPredict, Prosigna, BCI) received Grade B

Table 3. Clinical practice guidelines for use of gene expression assays to guide adjuvant chemotherapy recommendations.

		NCCN[48]		ASCO[49–51]			ESMO[52]		
Assay	**Population**	**Preference**	**Category of Evidence**	**Use**	**Level of Evidence**	**Strength of rec.**	**Use**	**Level of evidence**	**Grade of rec.**
Oncotype DX	N0	Preferred	1	Yes	High	Strong	Yes	I	A
	N1	Preferred (post)	1	No	Intermediate	Moderate	Yes	I	A
		Other (pre)	2A						
MammaPrint	N0	Other	1	Yes	High*	Strong	Yes	I	A
	N1	Other	1	Yes	High*	Strong	Yes	I	A
Prosigna	N0	Other	2A	Yes	High	Strong	Yes	I	B
	N1	Other	2A	No	Intermediate	Moderate	Yes	I	B
EndoPredict	N0	Other	2A	Yes	Intermediate	Moderate	Yes	I	B
	N1	Other	2A	No	Insufficient	Moderate	Yes	I	B
Breast Cancer Index	N0	Other	2A	Yes	Intermediate	Moderate	Yes	I	B
	N1	Other	limited	No	Insufficient	Strong	Yes	I	B

*High clinical risk only.
ASCO, American Society of Clinical Oncology; ESMO, European Society for Medical Oncology; ESMO grade of recommendation A, strongly recommended; ESMO grade of recommendation B, moderately recommended; ESMO level of evidence I, at least one large randomized, controlled trial of good quality, or meta-analysis of well-conducted randomized trials without heterogeneity; N0, node negative; N1, 1-3 involved nodes; NCCN, National Comprehensive Cancer Network; post, postmenopausal; pre, premenopausal; NCCN category of evidence 1, based on high level evidence, uniform NCCN consensus; NCCN Category 2A, based on lower level evidence, uniform NCCN consensus.

recommendations (strong or moderate evidence of efficacy, limited clinical benefit, moderately recommended).[52]

The NCCN guidelines, which were last updated in April 2021, endorse the Oncotype DX assay as their preferred genomic test, due to its ability to predict benefit from chemotherapy.[48] NCCN guidelines recommend that clinicians strongly consider utilizing the Oncotype DX assay in patients with HR+, HER2-negative N0 ductal, lobular, mixed, or micropapillary tumors measuring over 0.5 cm, and state that other assays, while prognostic, have not been validated to predict chemotherapy benefit. These guidelines are based on Level 1 evidence for Oncotype DX and MammaPrint, and on Level 2A evidence (based on lower-level evidence, uniform panel consensus) for other assays. In the N1 setting, the NCCN also recommends that clinicians strongly consider using the Oncotype DX assay for postmenopausal patients with ductal, lobular, mixed, or micropapillary carcinoma who are chemotherapy candidates, based on Level 1 evidence for Oncotype and MammaPrint, and Level 2A evidence for other assays, In the premenopausal setting, the NCCN recommends consideration of genomic testing for patients who are candidates for systemic chemotherapy, based on Level 1 evidence for MammaPrint, and level 2A evidence for other assays.[48]

As of yet, no guidelines have endorsed the utilization of the BCI assay to determine duration of adjuvant ET. While the NCCN guidelines describe the assay as predictive of EET benefit, they do not actively recommend its utilization,[53] and ASCO guidelines recommend against using gene expression assays to determine duration of ET.[51]

Discussion

Incorporation of multiparameter gene expression assays into clinical practice has altered treatment paradigms for early HR+ HER2-negative BCA, allowing the majority of postmenopausal women with up to 3 positive axillary lymph nodes to avoid cytotoxic therapy. Clinical validity has been well established for a number of commercially available gene expression assays for providing prognostic

information for recurrence; however, these assays are not interchangeable with regard to the prognostic information they provide. Direct comparison of several assays, including RS, MammaPrint, and ROR in the OPTIMA prelim trial showed that under 40% of tumors were classified similarly by all tests.[54] Comparison of RS, ROR, BCI, and EPclin scores in tumor specimens from women treated on the ATAC trial have shown that the latter 3 assays provide additional prognostic information compared with RS, which may be due to increased sensitivity to late relapse, and to the incorporation of clinical information into the ROR and EPClin algorithms.[55] The evaluation of the molecular mechanisms underlying these differences found that variations in RS were most strongly associated with changes in its module of estrogen-related genes, but that variations in the RS's proliferation module was most strongly correlated with changes in the ROR, BCI, and EPclin scores.[56] In addition, while these gene expression assay are all prognostic for recurrence, the RS is currently the only assay that has been shown to be predictive of chemotherapy benefit.[15] The RxPONDER trial demonstrates prospectively that RS can also be utilized to guide chemotherapy decision-making in patients with N1 disease,[18] although it remains unclear how much of the chemotherapy benefit seen in premenopausal women with RS 0-25 is actually due to premature menopause associated with chemotherapy use.[16] The incorporation of clinical information with RS, as in the RSClin online tool for those with negative axillary nodes, allows further personalization of prognosis and prediction to inform shared chemotherapy decision-making.[42] The ongoing OPTIMA trial will evaluate the ability of the ROR score to predict chemotherapy benefit, both in patients with N0 disease, and in those with up to 9 involved axillary nodes.[30]

Conflict of interest statement

The authors declare that there is no conflict of interest.

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