

Selecting postoperative adjuvant systemic therapy for early-stage breast cancer: An updated assessment and systematic review of leading commercially available gene expression assays

David M. Hyams MD¹  | Avital Bareket-Samish PhD² |
 Juan Enrique Bargallo Rocha MD³ | Sebastian Diaz-Botero MD⁴ |
 Sandra Franco MD⁵ | Debora Gagliato MD⁶ | Henry L. Gomez MD, PhD⁷ |
 Ernesto Korbenfeld MD⁸ | Gabriel Krygier MD⁹ | Andre Mattar MD¹⁰ |
 Aníbal Nuñez De Pierro MD¹¹ | Manuel Ruiz Borrego MD¹² | Cynthia Villarreal MD¹³

¹Medical Director, Desert Surgical Oncology, Eisenhower Medical Center, Rancho Mirage, California, USA

²Biolinsight Ltd., Binyamina, Israel

³Breast Cancer Department, Instituto Nacional de Cancerología Mexico and Centro Medico ABC, Mexico City, Mexico

⁴Breast Surgical Oncology Unit, Cancer Center at Clínica Universidad de Navarra, Madrid, Spain

⁵Medical Director, Centro de Tratamiento e Investigación sobre el Cáncer, CTIC, Bogotá, Colombia

⁶Department of Clinical Oncology, Beneficência Portuguesa de São Paulo, São Paulo, Brazil

⁷Breast Unit Director, OncoSalud, Clínica Delgado, AUNA, Universidad Ricardo Palma, Universidad Peruana Cayetano Heredia, Lima, Peru

⁸Department of Oncology, Hospital Británico de Buenos Aires, Buenos Aires, Argentina

⁹Department of Oncology, University Hospital de Clínicas, Montevideo, Uruguay

¹⁰Director of Mastology Center, Centro de Referência da Saúde da Mulher, Hospital da Mulher, São Paulo, Brazil

¹¹Department of Surgery, Unit of Mastology, Hospital J.A. Fernandez, Buenos Aires City, Argentina

¹²Medical Oncology Service, Hospital Universitario Virgen del Rocío, Seville, Spain

¹³Head, Department of Medical Oncology, Breast Cancer Center, Hospital Zambrano Hellion TecSalud, Tecnológico de Monterrey, Monterrey, Nuevo Leon, Mexico

Correspondence

David M. Hyams, MD, Desert Surgical
 Oncology Eisenhower Medical Center, Suite
 K-207, 39000 Bob Hope Drive, Rancho Mirage,
 CA 92270, USA.
 Email: Dhyams.md@me.com

Abstract

Gene expression assays (GEAs) can guide treatment for early-stage breast cancer. Several large prospective randomized clinical trials, and numerous additional studies, now provide new information for selecting an appropriate GEA. This systematic review builds upon prior reviews, with a focus on five widely commercialized GEAs (Breast Cancer Index[®], EndoPredict[®], MammaPrint[®], Oncotype DX[®], and Prosigna[®]). The comprehensive dataset available provides a contemporary opportunity to assess each GEA's utility as a prognosticator and/or predictor of adjuvant therapy benefit.

KEYWORDS

adjuvant chemotherapy, adjuvant drug therapy, biomarkers, breast neoplasms, clinical decision support systems, gene expression, prognosis

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Journal of Surgical Oncology* published by Wiley Periodicals LLC.

1 | INTRODUCTION

The use of multiplexed gene expression assays (GEAs) to assess risk and select therapy for early-stage breast cancer (ESBC) has become a modern standard of care.¹⁻⁸ Validation of GEAs has consisted largely of prospective/retrospective studies using annotated tissue samples from prior clinical trials. Only two commercialized GEAs have been subjected to true prospective clinical trials.⁹⁻¹² The current systematic review updates a prior review we published in 2017 designed to facilitate GEA selection for postoperative adjuvant therapy decision-making.¹³

Since then, consideration of GEAs in the neoadjuvant setting has increased.¹⁴ Some have suggested that GEAs be used to optimize preoperative tumor treatment. Others have suggested that tumor response to neoadjuvant therapy could be a surrogate endpoint for assessing a GEA's ability to predict long-term therapeutic benefit. Although response to preoperative treatment may foretell better outcomes for patients having pathologic complete response (pCR), it is well-recognized that comparisons of pCR are not directly translatable into equivalent long-term survival advantage.¹⁵ Therefore, we have chosen not to include neoadjuvant GEA studies with short-term response endpoints in this review.

There has been increasing interest in better selecting candidates for extended endocrine therapy (EET). As EET represents a relevant component of postoperative adjuvant therapy, we have included GEA publications relevant to such decision-making in this review.^{16,17}

2 | MATERIALS AND METHODS

2.1 | Article sources

Among the six assays previously assessed, IHC-4 has never been widely commercialized.¹³ Thus, it is excluded from the current review. For the remaining five GEAs (Table 1), we conducted a systematic search of the scientific literature focusing on primary published studies describing the ongoing clinical validation and utility of these tests. Databases searched included PubMed and the Cochrane Library. In addition, relevant previously published review articles were consulted to ensure that appropriate reports were not overlooked.

2.2 | Search strategy

A keyword search strategy was implemented that included "BCI", "Breast Cancer Index", "HOXB13/IL17BR", "MGI", "Molecular Grade Index", "Oncotype", "21 gene", "Recurrence Score", "MammaPrint", "70 gene", "PAM 50", "Prosigna", "EndoPredict", and "EPIClin". For each of the GEAs, the various names of the assay were searched, and the search was limited to breast cancer (BC). The search dates were from September 1, 2016 through March 1, 2023.

2.3 | Study selection and data extraction

Only English-language publications were selected. The analyzed studies included original research articles with a formal statistical test of pre-specified outcome endpoints. Studies in which the commercial GEA was not used and assay results were recapitulated from gene expression data measured through an RNA profiling platform were excluded, as were epidemiological, exploratory, decision impact, and health economics studies. Also excluded were studies without clinical outcomes or with a short follow-up (<3 years). We excluded neoadjuvant studies, publications unrelated to invasive BC, as well as most podium presentations unaccompanied by a subsequent peer-reviewed publication. Podium presentations that represented updates of previously peer-reviewed and published prospective trials were the only exception and are so indicated. Final reports published in E-format were eligible. All articles were fully reviewed by the investigators to determine that the study met the prespecified requirements. The PRISMA flow diagram for this review is presented in Figure S1.

2.4 | Study analysis

A total of 1053 articles were identified for inclusion in this analysis. Although several systems for assessing evidence quality have been proposed, the Tumor Marker Utility Grading System (TMUGS) remains an internationally accepted assessment tool.¹⁸ For consistency with our prior review, TMUGS was used to evaluate the level of evidence (LOE) of the identified studies.

3 | RESULTS

3.1 | Breast cancer Index® (BCI)

BCI is a multi-gene quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay combining the HOXB13:IL17BR ratio (H/I) and the Molecular Grade Index (MGI), each with independent prognostic value.^{7,19,20} The development and initial validation of the assay utilizing the two treatment arms of the Stockholm trial adjuvant tamoxifen (TAM) are described elsewhere.^{7,13,16,21} The commercially adopted BCI linear analytic model (BCI-L) had suggested that the MGI was more prognostic during the first 5 years, and the H/I ratio more robust after 5 years. More recently, Zhang¹⁶ and colleagues used the BCI-L to evaluate early (≤ 5 years) and late (> 5 -10 years) prognostic capability in the Stockholm population of 317 lymph node negative (LN-) patients who received tamoxifen (TAM+) without chemotherapy (CT). A second, somewhat higher-risk, set of 358 estrogen receptor positive (ER+), LN- patients was analyzed in the same publication. Individuals who were recurrence-free at 5 years were stratified to low-, intermediate-, or high-risk categories. Cox proportional hazards analysis of the late distant recurrence-free survival (DRFS) interval revealed a separation of the survival curves

TABLE 1 GEA summary for HR+ ESBC.

Characteristic	BCI	EndoPredict	MammaPrint	Oncotype DX	Prosigna
Manufacturer	Biotheranostics	Myriad Genetics	Agendia BV	Exact Sciences ^a	NanoString, Inc.
Methodology	RT-PCR	RT-PCR	Microarray	RT-PCR	nCounter system (NanoString)
Genes included in the assay	HOX B13/IL17BR ratio, molecular index (5 genes)	EP: 11 genes (8 cancer-related, 3 reference genes) EPclin: As EP plus nodal status and tumor size	70 genes	21 genes (16 cancer genes, 5 reference genes)	58 genes (50 PAM 50-related and 8 reference genes). The categorization also depends on nodal status.
Validated prognostic population	Premenopausal ✓ Postmenopausal ✓ LN- ✓ LN+✓	Premenopausal – Postmenopausal ✓ LN- ✓ LN+✓	Premenopausal ✓ Postmenopausal ✓ LN- ✓ LN+✓	Premenopausal ✓ Postmenopausal ✓ LN- ✓ LN+✓	Premenopausal ✓ Postmenopausal ✓ LN- ✓ LN+✓
Data for overall prognosis	✓	✓	✓	✓	✓
Data for late prognosis (beyond 5 years)	✓	✓	–	✓	✓
Predictor of CT benefit	–	–	–	✓	–
Predictor of ET benefit	✓ (Extended ET)	–	–	–	–

Abbreviations: BCI, Breast Cancer Index; CT, chemotherapy; ER, estrogen receptor; ESBC, early-stage breast cancer; ET, endocrine therapy; GEA, gene expression assay; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; LN, lymph node; RT-PCR, reverse transcriptase-polymerase chain reaction; TAM, tamoxifen.

^aDeveloped by GHI, which is now a wholly owned subsidiary of Exact Sciences.

for the three risk-groups, with differing statistical significance in the two study populations. BCI's ability to separate patients who were recurrence free at 5 years into binary high- and low-risk late recurrence groups was more robustly demonstrated in a 2017 reanalysis by Schroeder et al. evaluating T1N0 patients.²² BCI classified 32% (77/237) as high-risk in the Stockholm cohort and 36% (75/210) as high-risk in the multi-institutional cohort. This binary classification was significantly associated with late DRFS.

The BCI assay had also been evaluated by Sgroi et al.²³ using RNA extracted by Genomic Health, Incorporated (GHI) in a subset of the ATAC adjuvant trial of postmenopausal women treated with TAM or anastrozole. The BCI-L model was studied in ER+, human epidermal growth factor receptor 2 negative (HER2-) patients, producing a score between 0 and 10. Pre-specified cut points separated individuals into low-, intermediate-, and high-risk categories. Assessments were made for the first 5 years as well as for years between 5 and 10. For LN- patients through 5 years, the low- and intermediate-risk patients had DR of 1.3% (95% confidence interval [CI]: 0.5–3.1) and 5.6% (95% CI: 2.9–10.5), respectively, with overlapping CIs. However, the high-risk group, comprising 16% of the total patient population, produced a significantly greater DR risk of 18.1% (95% CI: 12.0–27.0). Between 5 and 10 years, the low-risk group, comprising 61% of patients, had a DR risk of 3.5%, separate from the overlapping DR risks of 13.4% (95% CI: 8.5–20.8) and 13.3% (95% CI: 7.5–23.4) for the intermediate- and high-risk patients, respectively.

The BCI transATAC population also included 250 LN+ patients classified into three prognostic risk groups for 10-year DR.²³ However, the prognostic value was not statistically significant in this population. As a result, Zhang et al.²⁴ proposed combining BCI with tumor size and grade (BCIN+). The integrated assay was validated in a retrospective analysis of 402 ER+, LN+ annotated patient samples from the Massachusetts General Hospital.²⁴ The heterogeneous population had endocrine therapy (ET), with 81% receiving some CT. In the validation, 81 patients were classified low-risk (20%) with a 15-year DR rate of 1.3% (95% CI: 0.0–3.7), versus 80% high-risk with a DR rate of 29% (95% CI: 23.2–34.4). Late recurrence after 5 years was 1.3% (95% CI: 0.0–3.7) and 16.1% (95% CI: 10.6–21.3), respectively.

Earlier studies had suggested that the H/I ratio was associated with response to TAM.^{25,26} In a prospective-retrospective nested case-control study of 249 patients treated with placebo or letrozole within the MA.17 trial, Sgroi²⁷ and colleagues had confirmed that elevated H/I was associated with lower late recurrence when letrozole EET was utilized. More recently, the predictive value of BCI was assessed in the setting of EET.^{28,29} Evaluation was performed through a prospective-retrospective study of patients enrolled in the phase III IDEAL trial of 1824 hormone receptor positive (HR+) postmenopausal patients.²⁸ Eligible individuals were randomized to receive either 2.5 or 5 years of aromatase inhibitor (AI) after completing 5 years of ET with TAM, AI, or a sequential combination. Nearly 73% of the translational cohort ($n = 908$) were LN+. The study simulated a randomized treatment/no-treatment trial

with a primary endpoint of recurrence-free interval (RFI) as a function of BCI risk assignment. For the overall cohort, BCI-high patients had recurrence rates of 5.9% (95% CI: 2.3–9.3) if randomized to 5 additional years of AI and 15.7% (95% CI: 9.5–21.5) if randomized to 2.5 additional years of AI. This represented a 9.8% absolute reduction in recurrence risk ($p = 0.011$). There was no significant risk reduction for BCI-low patients ($p = 0.84$). Even when the primary treatment (0–5 years) utilized an AI, BCI-high patients had an absolute recurrence benefit from EET of 11.8% ($p = 0.004$).

A second large prospective-retrospective study evaluated BCI prediction in the EET setting using TAM.²⁹ This was based on the aTTom trial, which randomized 6953 patients to 5 or 10 years of TAM, with improved outcomes for the longer regimen. Archival samples were centrally assessed for hormone receptor status and BCI results, with 56% of 2445 patients LN-, 32% LN+, and 12% uncertain. Due to the low LN- event rate, preplanned analysis was conducted in 789 patients from the LN+ subset. A significant RFI advantage was noted for TAM EET among BCI-high patients (hazard ratio [HR]: 0.33; 95% CI: 0.14–0.75; $p = 0.016$), with no significant benefit among BCI-low (HR: 1.11; 95% CI: 0.76–1.64; $p = 0.58$).

There is no high LOE data to suggest BCI predicts CT benefit.

3.2 | EndoPredict®

The EndoPredict assay (EP) was developed using microarray gene expression data from 964 ER+, HER2-, LN- tumors among TAM+ patients.⁵ Expression of eight cancer-related genes and three standardization genes by RT-PCR produces a continuous risk measurement. A pre-defined cut point separates high- and low-risk groups. The EP result, combined with nodal status and tumor size, produces the EPclin score. Details of the development and validation of this assay are summarized elsewhere.^{13,30} The primary validation studies were prospective-retrospective, meeting TMUGS standards for level IB evidence.^{5,18,31–33} Populations studied were primarily from the ABCSG-6 and ABCSG-8 clinical trials, as well as a group of patients enrolled in the GEICAM 9906 study of premenopausal patients, all of whom received CT. The GEICAM study design precluded evaluation of differential CT benefit as a function of EPclin risk assessment.³¹

With average increased follow-up of ASBCSG-8 and ASBCSG-6 for 4.2 years, a separate assessment of EPclin for LN+ patients became possible, as well as enhanced evaluation of the total population.³⁴ Of 1702 evaluable patients, EPclin classified 1066 (63%) as low-risk and 636 (37%) as high-risk. EPclin risk group significantly correlated to outcome, with the low-risk group having an improved DR-free rate (DRFR) over high-risk patients (HR: 4.8; 95% CI: 3.4–6.7; $p < 0.0001$). Multivariable analysis continued to show that EP retained significant prognostic value, although with a diminished HR (HR: 1.2; 95% CI: 1.1–1.3; $p < 0.0001$). EPclin's categorical separation of patients into low- and high-risk groups was confirmed for LN- patients (HR: 3.5; 95% CI: 2.2–5.6; $p < 0.0001$), for all LN+ patients (HR: 4.7; 95% CI: 2.3–9.7;

$p < 0.0001$), as well as for LN+ patients limited to 1–3 positive nodes (HR: 3.7; 95% CI: 1.7–7.7; $p = 0.0003$).

Because of increasing interest in EET, an extended analysis of late recurrence (from 5 to 15 years) was performed.^{34,35} Among 1386 patients who were DR-free at 5 years, a categorical low-risk EPclin score was associated with a lower risk of late DR (between 5 and 15 years) than a high-risk score (HR: 4.5; 95% CI: 2.7–7.7; $p < 0.0001$) over the same time period. These findings were consistent and statistically significant in evaluated subsets, including the LN-population, the entire LN+ population, and the LN+ population limited to 1–3 positive nodes.

EPclin has not been studied in any trials with randomized CT treatment/no-treatment arms. Soliman³⁶ and colleagues addressed this issue through complex modeling efforts using published data from the Early Breast Cancer Trialists Collaborative Group (EBCTCG). They proposed that ET-treated patients with low long-term risk would not be candidates for CT, whereas higher-risk patients would have greater absolute benefit, assuming a constant relative CT advantage. Any interaction between a gene-expression profile and CT benefit would be a minor consideration. Their mathematical model assessed the impact of fixed average CT benefit from meta-analyses of EBCTCG patients randomized in CT/no-CT polychemotherapy trials before 1995. They determined a relative risk reduction of CT of 30%, testing that value, as well as 20% and 40% values, using 10-year recurrence data from ABCSG-6 and ABCSG-8. Any separation between the DR curves, based on the *calculated* addition of CT versus ET-only in the EPclin-measured low-risk group, was small and clinically irrelevant. For EPclin high-risk patients, the *calculated* absolute benefit of CT increased with EPclin score and predicted patient risk. A variable “interaction” factor was included to represent any biological interaction between EPclin and CT benefit, but was not based on biological evidence.

An evaluation by Sestak utilized study populations from five previously analyzed prospective clinical trials, including ABCSG-6, ABCSG-8, transATAC, GEICAM 2003/2, and GEICAM/9906.³⁷ The study of 3746 women compared 2630 who received 5 years of ET alone from the ABCSG-6 and ABCSG-8 studies, and transATAC, with 1116 patients who received ET and CT from the two GEICAM studies. Although the overall population included pre- and postmenopausal women with HR+, HER2- disease, none of the patients who received ET alone were premenopausal, while 51% of the CT-treated patients were premenopausal. The groups also differed in a skew toward higher T-stage and nodal involvement in the GEICAM population. The authors evaluated 10-year risk of DR as a continuous function for both EP and EPclin. With increased score there was an increase in DR risk that was greater for ET alone versus ET with CT. The larger the score, the larger the separation between the two treatment groups. This was reflected in the interaction test between EPclin and CT with a $p = 0.022$. However, the same analysis of interaction using EP alone produced an insignificant p -value of 0.17, despite EP being prognostic for DR in the ET group (HR: 1.3; 95% CI: 1.2–1.3; $p < 0.0001$) and the ET + CT group (HR: 1.22; 95% CI: 1.2–1.3; $p < 0.0001$). This suggests that BC tumor size and nodal

status, recognized to have an association with both risk and relative CT benefit, may be the drivers for the significant interaction seen with EPclin.³⁷ The lack of a study directly comparing outcomes in a CT plus ET arm versus an ET alone arm leaves EP and EPclin without high-LOE for CT prediction.

3.3 | MammaPrint®

MammaPrint is a microarray-based assay that evaluates expression of 70 genes in tumor samples from patients with ESBC independent of HR-status, HER2 expression, or nodal status, and classifies patients into low or high genomic risk. The development and initial validation of the assay utilized annotated samples from the Netherlands Cancer Institute (NKI) and the translational Breast International Group (TRANSBIG) archive.^{1,38,39} MammaPrint was subsequently validated for prognosis in the prospective phase III MINDACT study (Figure S2), making it one of only two assays with TMUGS class I-A evidence.^{9,18} The 5-year distant metastasis-free survival (DMFS) among the primary test population of 644 clinical high-risk/genomic low-risk (cHgL) patients was 94.7% (95% CI: 92.5–96.2) without CT. This exceeded the study's predetermined noninferiority boundary of 92%.⁹ A recently updated analysis, with median follow-up of 8.7 years, yielded similar results.⁴⁰

MINDACT included a population of cHgL patients who were randomized to CT ($n = 749$) or no CT ($n = 748$). Among the intention-to-treat (ITT) individuals, 8-year DMFS was 92.0% (95% CI: 89.6–93.8) for CT and 89.4% (95% CI: 86.8–91.5) for the no-CT arm (HR: 0.66; 95% CI: 0.48–0.92), suggesting a nonsignificant DMFS trend to CT in this population.⁴⁰ The updated MINDACT publication also evaluated CT benefit in 1358 cHgL patients who were HR+ and HER2-. Among the 699 LN- patients, there was an 8-year DMFS absolute gain of 2.5% (SE: 2.3%; 95% CI: -2.1–7.2) for CT. Among the 658 LN+ patients the CT benefit was 1.3% (SE: 2.4%; 95% CI: -3.5–6.1). However, any advantage appeared limited to the 454 women ≤ 50 who received CT, with an 8-year DMFS of 93.6% (95% CI: 89.3–96.3) versus 88.6% (95% CI: 83.5–92.3) for no CT. Among the 690 cLgH patients, those who were randomized to CT had 8-year rate of survival without distant metastasis of 92.3% (95% CI: 88.7–94.8), compared to 90.8% (95% CI: 86.9–93.6%) for no CT.

Multifocal tumors were evaluated retrospectively using MINDACT case report forms. Among clinical low-risk (cL) patients, those with multi-focal disease were more likely to be classified as genomic high-risk (gH) (22.7%) versus a gL risk assignment (17.3%) (odds ratio [OR]: 1.45; 95% CI: 1.02–2.1; $p = 0.038$). Yet, multifocality was not associated with a change in DMFS.⁴¹

An exploratory analysis was performed in MINDACT focusing on a new ultralow genomic risk (gUL) category based on analyses of archived tumors from prior sources and studies (NKI, TRANSBIG, the RASTER trial, and the randomized Stockholm TAM trial).^{42,43} In the TRANSBIG cohort, 5/302 patients were classified as gUL with 15-year breast cancer-specific survival (BCSS) of 100% compared to 89.3% (95% CI: 82.6–96) for the 106 gL patients and 65.5% (95% CI:

57.5–73.5) for the 191 gH patients.⁴² In the Stockholm trial, cL patients were randomized to TAM versus no-treatment. The 54 gUL patients in the TAM arm had 20-year BCSS of 97%, versus 94% for the 44 gUL patients in the no-treatment arm, suggesting that sufficiently low-risk individuals might forego ET.⁴³ Multivariate analysis of 20-year breast cancer-specific mortality (BCSM), identified significantly higher patient risk for gL versus gUL (HR: 4.5; 95% CI: 1.4–14.8).⁴³

The MINDACT exploratory analysis used MammaPrint to reclassify 6693 patients into gUL ($n = 1000$, 15%), gL ($n = 3295$, 49%), and gH ($n = 2398$, 36%) risk groups.⁴⁴ Nearly 74% of gUL patients were classified as cL. Overall, 84% of gUL received adjuvant systemic therapy. The 8-year distant metastasis-free interval (DMFI) was 97.0% with a significant DMFI advantage for gUL versus gL (HR: 0.65; 95% CI: 0.45–0.94). Another exploratory analysis compared untreated gUL patients to those who received ET alone, with excellent outcomes in both (8-year DMFI: 98% and 97%, respectively).⁴⁴

A prospective-retrospective analysis by Opdam⁴⁵ and colleagues evaluated the gUL classification with postmenopausal patients from the Netherlands Cancer Institute IKA trial, randomized to no-treatment versus 1–3 years of TAM. Again, gUL patients had an excellent outcome, supporting the findings of the initial gUL validation in the Stockholm trial and MINDACT. Nonetheless, unresolved questions remain regarding the effect of withholding or decreasing ET on very long-term outcome.^{43,45} The gUL classification has also been examined in additional lower LOE analyses, producing broadly consistent results.^{46,47}

A prospective-retrospective analysis by Dubsky⁴⁸ and colleagues utilized the biomarker cohort of the ABCSG-8 study to evaluate MammaPrint. Of 1347 ER+ ET-treated patients, only 658 (49%) could be included due to RNA degradation in the formalin-fixed, paraffin-embedded (FFPE) samples. MammaPrint classified 78% as gL and 22% as gH, and was shown to be a statistically significant prognosticator in the analysis (5-year DRFS 94.0% versus 91.6%; 10-year DRFS 91.3% versus 84.8% in the gL versus gH groups, respectively). However, the multivariable analysis only demonstrated statistically independent prognostication at 5 but not 10 years.

Vliek⁴⁷ and colleagues recently reported 10-year data on the ER+ HER2- subset of patients ($n = 310$) from the prospective observational RASTER study.⁴⁹ Some 162 (52%) were classified as cLgL, 56 (18%) as cLgH, 45 (15%) as cHgH, and 47 (15%) as cHgH. Treatment decisions were made jointly by the patient and the treating physician taking into account clinicopathologic characteristics, personal preferences, the MammaPrint results, and contemporaneous treatment guidelines. Distant recurrence-free interval (DRFI) at 10 years for patients classified as cHgH was excellent (95%) regardless of CT use.⁴⁷ Despite the limitations of this low-LOE nonrandomized study with heterogeneous treatment regimens, the RASTER analysis suggests that real-world observational data is consistent with the MINDACT study. However, like MINDACT, it provides no evidence to support a role for MammaPrint as a predictor of CT benefit.⁴⁷

3.4 | Oncotype DX[®]

The Oncotype DX assay is an RT-PCR-based 21-gene assay that was designed to assess DR risk in ER+ ESBC. A proprietary algorithm produces a continuous Recurrence Score[®] (RS) result from 0 to 100, with low (0–17), intermediate (18–30), and high-risk (31–100) categories. Alternative risk cut points have been proposed and increasingly utilized.^{11,12,50} Validation was performed in a series of prospective-retrospective studies of archived tissue samples from large clinical trials of ESBC. Studies included HR+ premenopausal and postmenopausal patients, as well as LN+ and LN- individuals, providing TMUGS level IB evidence. Details have been reviewed elsewhere.^{13,51} Notable were two studies that included adjuvant ET, with a randomized comparison of CT versus not.^{52,53} These provided high LOE support for the Oncotype DX assay as a predictor of CT benefit only for N0 or N1 patients with elevated RS results. Application of these findings has resulted in a major change in the proportion of HR+ ESBC patients who use adjuvant CT.^{54–69}

The Oncotype DX assay has been evaluated in three fully prospective trials. These include: (1) TAILORx: a study of ER+, HER2-, N0 ESBC patients, (2) RxPONDER: a trial of HR+, HER2-, N1 patients, and (3) The West German Study Group (WSG) PlanB: a trial of HR+, HER2-, N0-3 patients.^{10–12,50,70–72} An additional prospective study, the WSG ADAPT utilized the RS result as part of a biologic decision matrix to assign treatment.⁷³ At the time of our prior review, only two (TAILORx and WSG PlanB) had reported outcome data, with a focus on each study's lowest-risk population. The respective investigators have since published complete primary endpoint data for TAILORx, PlanB, and RxPONDER.

TAILORx and RxPONDER assigned a new cut point for high-risk (RS > 25). TAILORx was designed to validate RS prognosis, while assessing prediction of CT benefit by randomizing patients with RS 11–25.^{10,11,70} RxPONDER prospectively evaluated RS prediction for CT benefit in N1 patients with RS 0–25.¹² The study designs for both trials are summarized in Table S1 and Figure S3. In TAILORx, patients with RS 0–10 received ET alone, while patients with RS 26–100 were to receive CT followed by ET (ET + CT). Midrange RS patients (11–25) were randomized (1:1) to ET versus ET + CT.^{10,11,70} In RxPONDER, all eligible patients were randomized to ET alone versus ET + CT. The primary objective was to determine whether ET + CT improved invasive disease-free survival (iDFS). Secondary objectives included an assessment of CT benefit as a continuous function of increasing RS from 0 to 25, as well as evaluation of the RS result as a prognostic indicator. Baseline characteristics of patients in TAILORx and RxPONDER are available in Table S2. In TAILORx, 10 273 LN- patients were assigned treatment, while 5083 LN+ patients were assigned treatment in RxPONDER.^{10–12,70}

Initial results of TAILORx were published in 2015, and subsequently updated in 2018 and 2019.^{10,11,70} The investigators provided a further update in abstract form in 2023.⁷⁴ With a median follow-up of at least 10.4 years for the overall population and 11 years for the randomized population, the study confirmed the prognostic capability of the Oncotype DX assay using all pre-

specified survival endpoints. Differentiation between RS categories in all the endpoints was highly significant ($p < 0.001$).

For patients with RS 0–10, clinical outcomes were excellent with ET alone.^{11,74} Multivariate analysis identified no impact of age, tumor size, or surgery type. The 12-year outcome data for this very low RS population included an iDFS rate ($\pm$ SE) of $75.9 \pm 1.3\%$, a DRFI rate of $93.2 \pm 0.8\%$, a RFI of $91.4 \pm 0.9\%$ with an overall survival (OS) of $89.8 \pm 0.8\%$.⁷⁴ The iDFS event rate for second BC and non-BC events exceeded primary BC recurrence events.

In the RS 11–25 arms, 12-year iDFS analysis identified no advantage of ET + CT ($77.4 \pm 0.9\%$) versus ET alone ($76.8 \pm 0.9\%$), confirming non-inferiority for the primary endpoint.⁷⁴ Non-inferiority for ET + CT was maintained for the remaining evaluated endpoints including DRFI, RFI, and OS. There was no interaction between CT treatment effect and RS subcategory (RS 11–15 vs. 16–20 vs. 21–25 or 11–17 vs. 18–25) or continuous RS value. There was no significant relationship between CT treatment and tumor size, grade, menopausal status, or clinical risk category.¹⁰ However, there was a significant interaction between CT benefit and age, with benefit observed for younger women ages within the RS range of 16–20 and 21–25. For RS 16–20 patients, the 9-year DFS HR for ET versus ET + CT 1.9 (95% CI: 1.3–2.8; $p = 0.0016$).¹⁰ For RS 21–25 patients, HR for the same comparison was 1.7 (95% CI: 1.03–2.8; $p = 0.035$). This represented a 1.6% 9-year DR advantage to CT for RS 16–20 and 6.5% for RS 21–25.

Additional analyses assessed clinical risk in women ≤ 50 at diagnosis.⁷⁰ Low-risk included tumor diameter ≤ 3 cm and grade 1, ≤ 2 cm and grade 2, or ≤ 1 cm and any grade. Remaining tumors were considered high-risk. The absolute CT benefit (DR at 9 years) was independent of clinical risk for RS 21–25, whereas CT benefit was only observed in high clinical risk patients ($6.5 \pm 4.9\%$) in the RS 16–20 subgroup.⁷⁰ Interestingly, a high RS result (> 25) was noted among 8.9% of patients with low clinical risk. This illustrates the value of the GEA in identifying not only patients for whom CT will not be of value, but also those for whom CT should be considered.⁷⁰

In RxPONDER there was no iDFS benefit evident for CT in the N1 RS ≤ 25 ITT population (5-year iDFS ET: 91.0% vs. CT + ET: 92.2%; $p = 0.1$).¹² There was also no DRFS improvement or significant interaction between CT and the numerical RS result, RS subcategory, or type of surgery. However, there was a significant interaction between CT benefit and menopausal status ($p = 0.008$). A statistically significant benefit in iDFS and DRFS was evident with CT among the 1665 premenopausal patients analyzed (5-year iDFS HR: 0.6; 95% CI: 0.43–0.83; $p = 0.002$). The 5-year iDFS rate was 89.0% with ET alone versus 93.9% with ET + CT, resulting in an absolute CT gain of 4.9%. Similar gains were noted for DRFS. The benefit of adding CT to premenopausal patients was consistent across most predetermined subgroups and RS sub-groupings. A recently presented analysis of premenopausal patients by the primary study group, found that ET + CT improved DRFI across the entire RS 0–25 range, with an absolute gain of 2.4%.⁷⁵

The WSG PlanB clinical trial, was updated by Nitz et al.⁷⁶ in 2019 with final results. This investigation was primarily designed as a

classic comparison of CT regimens in high-risk ESBC patients. As presented in our earlier review, 348 patients with RS ≤ 11 and ET alone had a 3-year DFS rate of 98.4% (95% CI: 97.0–99.8), despite 31.4% having LN+ and 20% having grade 3 disease.¹³ The updated publication focused on the CT comparison which identified no advantage to either anthracycline-based CT or a taxane-cyclophosphamide-based CT.

Unlike TAILORx, RxPONDER, and WSG PlanB, WSG ADAPT HR+/HER2– study was a prospective open-label phase II/III trial combining Ki-67 to assess brief preoperative ET with RS result as a static biomarker.⁷³ A 386-patient run-in phase was followed by the actual trial.⁷⁷ HR+ HER2– ESBC patients with ≤ 3 positive nodes underwent 3 weeks of preoperative ET treatment bookended by two biopsies to assess tumor response defined as Ki-67 $\leq 10\%$. Patients received adjuvant ET if the RS result was 0–11 (control arm, $n = 868$), or if their RS result was 12–25 and they were ET responders (experimental arm, $n = 1422$). Non-responder RS 12–25 patients, and patients with RS 26–100, received dose-dense CT + ET.⁷³ ET response decreased as the RS result increased (78.8%, 62.2%, and 32.7% for RS 0–11, 12–25, and 26–100, respectively, $p < 0.001$), and was greater with AI than TAM. The 5-year iDFS was 92.6% (95% CI: 90.8–94.0) in the experimental versus 93.9% (95% CI: 91.8–95.4) in the control arm confirming non-inferiority. In contrast, 5-year iDFS in the 694 ET nonresponder patients with RS 12–25 who received CT was 90.3% (95% CI: 87.2–92.6). Similar outcomes were noted in distant disease-free survival (dDFS).⁷³

The NSABP B-20 trial comparing TAM versus TAM plus CT, provided the first level 1B evidence for the Oncotype DX assay.⁵³ A recent re-analysis of B-20 by Geyer⁷⁸ and colleagues excluded HER2+ patients and assessed CT benefit using both the original RS cutoff values as well as the newer TAILORx cutoff values. Among 569 of 651 patients (87%) included in the original validation trial, CT benefit remained for patients with RS 31–100 (HR: 0.18; 95% CI: 0.07–0.46). A less robust benefit was noted in patients with an expanded high-risk RS 26–100 (HR: 0.27; 95% CI: 0.12–0.62). There were too few samples to analyze the specific interval RS 26–30.⁷⁸

Retrospective-prospective analysis of NSABP B-28 included 1065 LN+ patients who received doxorubicin-cyclophosphamide (AC) +/- subsequent paclitaxel treatment.⁷⁹ The RS result was a significant predictor of outcome for all evaluated endpoints with a median follow-up of 11.2 years, but did not predict the added benefit of CT regimen.⁷⁹ However, the RS result was a significant and independent predictor of locoregional recurrence (LR) (10-year LR of 3.3%, 7.2%, and 12.2% for RS 0–17, 18–30, and 31–100, respectively; $p < 0.001$).⁸⁰ A similar analysis of the PACS-01 study included 530 LN+ patients who received CT +/- subsequent docetaxel treatment, followed by ET.⁸¹ Again, RS stratified outcome, but failed to predict benefit of docetaxel.

Additional retrospective-prospective analyses of the B-14 (LN–, TAM-only) and B-28 (LN+, TAM + CT) studies assessed late (> 5 years) DR as a function of estrogen receptor gene (ESR1) expression and tumor proliferation.⁸² Using a B-28 derived quantitative ESR1 expression cut point, among the 86% of B-14 patients with high

ESR1 expression, the RS result significantly predicted late DR (HR for a 50-point RS difference, 2.2; 95% CI: 1.1–4.5; $p = 0.04$).⁸² The authors suggested that low ER-expressing tumors, as well as highly proliferative high ER-expressing tumors, fail early (≤ 5 years) while low-proliferation high ER-expressing tumors have a more significant late failure rate.

To further integrate clinical features, a recent meta-analysis including 10 004 LN- patients participating in B-14 and TAILORx was used to develop a new tool (RSClin[®]) designed to integrate the RS result with age, tumor grade, tumor size, and ET type.⁸³ The RSClin tool, subsequently validated in an external cohort from the Clalit registry, provides estimates for 10-year DR and absolute CT benefit for LN- HR+ HER2- ESBC patients.⁸³

Of the hundreds of recent studies evaluating the impact of the Oncotype DX assay on clinical practice, 73 were retrospective cohort studies. These studies reflect real-world data and have used single-/multi-center cohorts to either validate the assay in unselected RS-tested patient populations or to focus on specific subgroups of interest. These studies plus other analyses that are beyond the scope of the main manuscript are summarized in Table S3. The studies were largely consistent with previous prospective and prospective-retrospective investigations. The largest of these retrospective studies were derived from the US Surveillance, Epidemiology, and End Results (SEER) database and the US National Cancer Database (NCDB).

Petkov⁸⁴ and colleagues linked RS results to the SEER database and identified 38 568 LN- and 4691 LN+ HR+ HER2- patients with RS results diagnosed between 2004 and 2011. Using BCSM and the three original cutoff values, the RS result was demonstrated to be a significant predictor of BCSM risk for LN- and LN+ patients separately and for all analyzed subgroups (age, grade, race, and socioeconomic status).⁸⁴ Three recent analyses of the SEER database including OS and BCSS data examined the benefit of CT in patients with tumors in the range of RS 26–30.^{85–87} Yang⁸⁷ and colleagues evaluated 3754 LN- ESBC patients, 58% of whom received adjuvant CT with RS 26–30. Using propensity score matching, CT was independently associated with better BCSS ($p = 0.016$). This was particularly evident in patients with high-grade tumors having adverse prognostic features.⁸⁷ Ma and associates studied 5054 SEER patients with T1-2N0 ESBC, RS > 26, using Kaplan–Meier, log-rank, and Cox multivariable analyses.⁸⁶ Using propensity score matching, and subgroups of RS 26–30 and RS > 30, adjuvant CT was associated with improved OS (HR: 0.66; 95% CI: 0.53–0.83; $p < 0.001$) and BCSM (HR: 0.61; 95% CI: 0.45–0.83; $p = 0.001$). Of 943 matched patients with RS 26–30, CT added significant benefit to OS (HR: 0.52; 95% CI: 0.34–0.79; $p = 0.003$) and BCSS (HR: 0.42; 95% CI: 0.22–0.81; $p = 0.009$). Park and colleagues performed a similar analysis using the SEER database, studying ESBC patients with RS 18–25 and 26–30.⁸⁵ They noted that patients with RS 26–30 who received CT (3130 of 5290) had a significantly decreased risk of BCSM (HR: 0.68; 95% CI: 0.44–0.99) and overall mortality (HR: 0.58; 95% CI: 0.44–0.76) after adjustment for demographic, clinicopathological, and treatment factors.

An analysis of the NCDB data by Ibraheem⁸⁸ and associates focused on 73 185 female BC patients with Stages I–IIIA BC. The authors examined the OS benefit of CT in 5930 N0 and 1176 N1-2a patients with RS 26–30. Adjusted HR for patients receiving CT was 0.68 (95% CI: 0.48–0.96) for LN- patients and 0.50 (95% CI: 0.28–0.89) for LN+ patients. The 5-year gains in OS with CT were 1.8% for the LN- and 6.7% for the LN+ patients. Although the benefit of CT in LN- patients was limited to RS 26–30 patients, among LN+ patients, statistically significant CT benefit was seen in all RS subgroups evaluated (11–17, 18–25, and 25–30). However, the benefit was nearly sixfold greater for the RS 26–30 subgroup versus the 11–17 subgroup.⁸⁸

Five studies based on the SEER database and one based on the NCDB database have since explored racial/ethnic differences in RS results and clinical outcomes.^{89–94} These included between ~67 000 and 88 500 patients focusing on non-Hispanic White (White), non-Hispanic Black (Black), and Chinese American patients. There was no ethnic group for which the RS result was found not to be prognostic, although in one study by Jung⁹⁰ and colleagues the ability of the assay to predict CT benefit was not confirmed in non-White women. Several studies identified an RS result shift in Black women towards higher values.^{90,92–94} In TAILORx, Black patients (4% of the TAILORx population) were noted to have poorer outcomes overall, with a disproportionately higher event rate during the first 5 years, but not during the subsequent years.^{74,95} The adverse impact of race was maintained, but modestly attenuated, when adjusted for age, tumor size, grade, RS, and insurance status. However, the overall lack of CT benefit for RS 11–25 was still observed for Black and White patients when analyzed separately.

3.5 | Prosigna[®]

The Prosigna assay is based on expression of 50 cancer genes (PAM-50) and eight reference genes utilizing the nCounter[®] Analytic System of Nanostring Inc. The Prosigna algorithm calculates a risk of recurrence (ROR) from gene expression data and tumor size, producing a 10-year DR risk. Prosigna also assigns a BC subtype based on the classification system initially described by Perou, et al.⁹⁶ The assay was developed and validated using a prospective-retrospective approach with ET-treated ER+, LN-/LN+, postmenopausal patients from the ABCSG-8 trial ($n = 1478$) and the transATAC study ($n = 1017$), providing TMUGS level IB evidence.^{3,4} The development and validation of the assay are described elsewhere.^{13,51,97} LN- patients may be low (ROR 0–40), intermediate (ROR 41–60) or high (ROR 60–100) risk; LN+ patients with 1–3 positive nodes may also be classified as low- (ROR 0–15), intermediate- (ROR 16–40) or high-risk (ROR 41–100), but all LN+ patients with ≥ 4 positive nodes are classified high-risk regardless of their ROR score.

Jensen has reported further prospective-retrospective analyses using FFPE tumor samples from prospective randomized Danish trials of high-risk early-stage BC. The first used samples from the 45-year-

old DBCG 77B trial, in which elevated-risk premenopausal patients received radiation therapy (RT) and were randomized to no-treatment, levamisole (considered no-treatment), cyclophosphamide (C), or C with methotrexate and fluorouracil (CMF).⁹⁸ ET was not used in HR+ patients.⁹⁹ Patients ($n = 460$) with available blocks and successful Prosigna assay results were mostly LN+ (87%) and HR+ (72%). Prosigna classification included luminal A (35%), luminal B (25%), basal-like (13%), and Her2-enriched (26%). In the no-CT group, continuous ROR score was highly prognostic (HR for DFS per 10-point difference in ROR 1.2; 95% CI: 1.1–1.4; $p < 0.001$). There was no statistically significant differential between early (0–5 years) and late (>5 years) prognostic ability of Prosigna in the no-CT group. For the C/CMF group, ROR was prognostic in years 0–5 only.⁹⁸ This analysis used prespecified ROR cutoff values independent of nodal status.⁹⁸ In the no-CT group, the 10-year DFS rates for the low-risk group differed significantly from the intermediate- and high-risk groups.

There was no effect of the continuous ROR score on treatment with C/CMF for DFS ($p_{int.} = 0.66$) or OS ($p_{int.} = 0.32$). Furthermore, there was no significant interaction ($p_{int.} = 0.11$) between ROR score (using the commercial low-risk ROR cut point) and CT in patients with ER+ HER2– BC. However, the interaction between Prosigna subtypes and treatment was significant for DFS ($p_{int.} = 0.003$) and OS ($p_{int.} = 0.04$).⁹⁸ The most pronounced CT benefit was in the basal-like subtype (unadjusted HR: 0.19; 95% CI: 0.09–0.40) followed by luminal B (unadjusted HR: 0.47; 95% CI: 0.28–0.77), and luminal A (unadjusted HR: 0.64; 95% CI: 0.36–1.1). No C/CMF benefit was observed for the Her2-enriched subtype.⁹⁸

The second study reported by Jensen et al.¹⁰⁰ utilized the DBCG 89D randomized trial of CMF versus C plus epirubicin and fluorouracil (CEF) in high-risk early-stage BC. In the Prosigna study population ($n = 686$), treatment benefit favoring CEF for DR was evident. These patients were mostly premenopausal (69%) and LN+ (64%), with 33% HER2+ and 23% ER+. None of the patients received ET or HER2-targeted therapy. Prosigna classified 19% as luminal A, 11% as luminal B, 38% as basal-like, and 32% as Her2-enriched. With a median follow-up of 9.8 years, continuous ROR score was prognostic in both treatment arms. ROR could not be evaluated as a predictor of CT benefit as all patients in the study received CT. However, there was no relationship between ROR and CT regimen (CMF vs. CEF) ($p_{int.} = 0.16$ for DR).¹⁰⁰ Prosigna subtype analysis revealed better DR and OS outcomes with CEF compared to CMF in the Her2-enriched subtype only (HR: 0.58; 95% CI: 0.38–0.86). Interaction between subtype and treatment effect was significant only for OS ($p_{int.} = 0.03$).¹⁰⁰

Fitzal¹⁰¹ and colleagues re-visited the ABCSG-8 trial to evaluate LR and radiotherapy benefit as a function of Prosigna result. Their analysis involved 1034 postmenopausal patients (70% LN–, 30% LN+) who were to receive RT after breast-conserving surgery. Patients were classified as low- or high-risk according to ROR, independent of nodal status. ROR as either a continuous or categorical variable was prognostic of LR. In particular, the unadjusted subhazard ratio (SHR) for high versus low-risk ROR was 4.8 (95% CI: 1.7–13.2; $p = 0.003$), remaining significant in a

multivariable analysis. Prosigna subtyping also provided prognostic information, but with all groups having low single-digit recurrence rates. Furthermore, SHRs for a 10-point difference in ROR were similar among patients who received RT versus those who did not, and the interaction was nonsignificant ($p_{int.} = 0.48$), indicating that ROR was not predictive of RT benefit.¹⁰¹

3.6 | GEAs in BC histologies beyond IDC

The clinical behavior of invasive lobular cancer (ILC) may be distinct.¹⁰² The GEAs discussed herein were also assessed in patients with ILC and other non-IDC histologies. These studies are summarized by GEA in Table 2. The evidence suggests that the clinical utility of the GEAs in IDC is overall similar to that in ILC and other non-IDC histologies.

3.7 | Cross-platform comparison

This review has focused on developmental and validation data supporting each individual GEA's prognostic and predictive capabilities. However, validation studies may have subtle intrinsic design biases that optimize the assay under study. Furthermore, some GEA's may have greater utility in differing sub-populations. Ideally, GEAs should be prospectively assessed against one another in randomized trials that evaluate their impact using standardized outcome measures. In such trials, the test should be performed by the relevant manufacturer (or designate) under real-world conditions that reflect the commercial norm. However, such studies are uncommon as they must be large, have long-term outcome data, and require commercial results data from relevant GEA manufacturers.

Several studies have attempted direct comparison. However, they rarely include tests performed under standard commercial and clinical conditions. In some cases they have used specimen processing performed “in-house” while in others they have had tissue prepared by competing commercial laboratories.^{114–116} In some cases they have used simulations designed to recapitulate competing commercial assays.^{117–123} In some studies, investigators have used cut-points and subgroup estimates which differ substantially from current commercial assays.^{124,125} Menopausal status definitions have varied between GEAs. ER-status and HER2 expression also varied between the relevant validation studies. All of this suggests the limits of ad hoc cross-platform comparison.

Most comparative studies may be sorted into two broad categories. The first includes studies that have examined discordance and concordance between GEAs. These are summarized in Table 3. The evaluations show only moderate agreement between the assays. The second group of studies are those that have utilized messenger RNA (mRNA) isolated from samples archived from the prospective transATAC trial. These studies, focusing on each assay's prognostic output compared to trial outcome data, are summarized in Table 4. In these analyses, likelihood ratios and c-statistics are often used to rank

TABLE 2 Summary of studies investigating ILC and other non-IDC histologies by GEA.

#	Author/reference	Study design, N	Patient population ^a	Main endpoint (outcomes-focused)	Main results (outcome-focused)
BCI					
1	Nunes et al. ¹⁰³	Retrospective cohort study, multi-institutional convenience sample, n = 307 ILC	All ER+ ILC BC 94% HER2- 58% LN-, 42% LN+ 93% ET-treated 50% CT-treated	Time to DR	BCI was an independent prognostic factor for ILC for high- vs. low/intermediate-risk patients, MVA: - Overall 10-year DR: HR, 3.5; 95% CI: 1.3-9.5; p = 0.015
EndoPredict (EPclin)					
1	Sestak et al. ¹⁰⁴	Prospective-retrospective with samples from 3 randomized phase 3 clinical trials (ABCSG-6, ABCSG-8, and ATAC) n = 470 ILC n = 1944 IDC n = 216 other histologies	All ER+ HER2- BC All postmenopausal ILC, 69%/31% LN-/LN+ IDC, 70%/30% LN-/LN+ All received only ET	10-year DR risk	In both the IDC and ILC populations, EPclin was a significant prognosticator. Among ILC patients, the HR for DR risk (high-risk vs. low-risk patients): - LN-: HR, 2.6; 95% CI: 1.6-4.0; p < 0.0001 - LN+: HR, 3.7; 95% CI: 2.5-5.5; p < 0.0001 Among the non-IDC non-ILC subtypes, EPclin was also a significant prognosticator. - All patients: HR, 2.4; 95% CI: 1.7-3.4; p < 0.0001
MammaPrint					
1	Beumer et al. ¹⁰⁵	Retrospective analysis of Agendia's clinical series database n = 217 ILC	All ILC 94% ER+ 92% HER2- 66% LN-, 34% LN+ 59% ET-treated 22% CT-treated	10-year outcomes (OS, DMFI, DMFS)	MammaPrint was an independent prognostic factor for all patients (except for 10-year OS) and in LN- patients separately. All patients, HRs for 10-year outcomes (high- vs. low-risk patients, MVA): - OS: HR, 2.0; 95% CI: 0.94-4.3; p = 0.07 - DMFI: HR, 2.4; 95% CI: 1.004-5.6; p = 0.049 - DMFS: HR, 2.1; 95% CI: 1.05-4.1; p = 0.037 LN- patients, HRs for 10-year outcomes (high- vs. low-risk patients, MVA): - OS: HR, 5.1; 95% CI: 1.5-17.1; p = 0.008 - DMFI: HR, 11.1; 95% CI: 2.3-53.0; p = 0.03 - DMFS: HR, 6.4; 95% CI: 2.1-19.2; p = 0.01
2	Abel et al. ¹⁰⁶	Retrospective cohort study using the NCDB n = 1497 ILC n = 5902 IDC	All ER+ HER2- ILC, 93% ET-treated, 29% CT-treated IDC, 91% ET-treated, 37% CT-treated	The rate of discordant clinical/genomic results 5-year OS	- More discordant patients (clinical/genomic) in ILC vs. IDC (47% vs. 37%, p < 0.001), and especially in cHgL patients (36% vs. 19%, p < 0.001). - In unadjusted/unmatched analysis, among cHgL cases who received CT, IDC patients had better OS vs. ILC patients (p = 0.02). In cLgH cases, there was no difference by histology.
3	Jenkins et al. ¹⁰⁷	Retrospective cohort study using the NCDB n = 2610 ILC	All ER+ HER2- ILC 75% LN-, 25% LN+ 94% ET-treated 18% CT-treated	5-year OS	MammaPrint was a significant prognosticator for 5-year OS. - HR (high vs. low genomic risk by MammaPrint), 2.1; 95% CI: 1.1-3.9; p = 0.03.

TABLE 2 (Continued)

#	Author/reference	Study design, N	Patient population ^a	Main endpoint (outcomes-focused)	Main results (outcome-focused)
Oncotype DX					
1	Kizy et al. ¹⁰⁸	Retrospective cohort study using the SEER database n = 7316 ILC	All ER+ ILC 85% LN-, 15% LN+ 20% CT-treated	5-year BCSS	The RS was a significant prognosticator: - The 5-year BCSS was 99% in RS 0–10, 99% in RS 11–25, and 96% in RS 26–100 patients (HR for RS 26–100 vs. RS 0–10 patients, 2.4; 95% CI: 1.1–5.0). CT benefit: - Among RS 11–25 patients, adjuvant CT was not associated with BC death risk (HR, 1.1; 95% CI: 0.62–1.9). - 77% of patients had RS 0–17, 23% had RS 18–30, and none had RS 31–100. - At 40 months, DRFS for the entire cohort was 100%.
2	Turashvili et al. ¹⁰⁹	Single-center retrospective cohort study (US) focusing on rare histologies n = 57 (33 mucinous carcinoma, 10 tubular carcinoma, 9 encapsulated papillary carcinoma, and 5 solid papillary carcinoma).	All ER+ HER2- 93% LN-, 7% LN+ 97% ET-treated 12% CT-treated	DRFS at 40 months	
3	Chen et al. ¹¹⁰	Retrospective cohort study using the SEER database n = 6467 ILC	All ER+ ILC 88% LN-, 12% LN+ 19% CT-treated	5-year BCSS	The RS was a significant prognosticator: - The 5-year BCSS was 99.4%, 98.1%, and 92.9% for RS 0–17, 18–30, and 31–100 patients, respectively ($p < 0.001$). In MVA, HR for 5-year BCSS for RS 31–100 vs. 0–17 patients, (HR, 2.9; 95% CI: 2.1–3.9; $p < 0.001$). CT benefit: - CT did not confer statistically significant BCSS benefit in any of the RS subgroups.
4	Makower et al. ¹¹¹	Retrospective cohort study using the NCDB n = 57 615 IDC n = 8693 ILC n = 5393 mixed IDC/ILC n = 2459 mixed IDC/other n = 312 mixed ILC/other	All ER+ HER2- Patient characteristics (ranges): LN-: 82%–87% LN+: 13%–18% ET-treated: 98%–99% CT-treated: 19%–27%	Time to event (OS)	- IDC patients were more likely than ILC patients to have RS 26–100 (16.9% vs. 7.2%, $p < 0.0001$) The RS was a significant prognosticator: - The RS was prognostic in IDC ($p < 0.0001$), ILC ($p = 0.0122$), mixed IDC/ILC ($p = 0.0023$) but not in IDC/other ($p = 0.49$). For ILC/other, the sample size was too small to perform the analysis. CT benefit: - CT benefit was not evaluated by RS category. - Overall, adjuvant CT was associated with improved OS in IDC (HR = 0.76, $p < 0.0001$) but not in ILC (HR = 0.99, $p = 0.93$), mixed IDC/ILC (HR = 1.04, $p = 0.86$), IDC/other (HR = 0.87, $p = 0.71$), or ILC/other (HR = 2.91, $p = 0.10$).
5	Oesterreich et al. ¹¹²	Multi-center retrospective cohort study (US) n = 2564 ^b ILC (609 with RS results) n = 17 724 ^b IDC (3457 with RS results)	ER+ subset ILC, 59%/41% LN-/LN+ IDC, 64%/36% LN-/LN+ ILC, 90% ET-treated, 41% CT-treated	Time to event (DFS, OS)	- IDC patients were more likely than ILC patients to have RS 26–100 (11% vs. 2%, $p < 0.001$) ^c The RS was a significant prognosticator for IDC and not ILC: - IDC: HR for DFS, RS 0–25–100 vs. RS 26–100 patients, 0.34, 95% CI: 0.22–0.53;

(Continues)

TABLE 2 (Continued)

#	Author/reference	Study design, N	Patient population ^a	Main endpoint (outcomes-focused)	Main results (outcome-focused)
6	Weiser et al. ¹¹³	Retrospective cohort study using the NCDB n = 15 763 ILC n = 100 070 IDC	IDC, 87% ET-treated, 41% CT-treated All hormone receptor+ and HER2- ILC, 83%/17% LN-/LN+ IDC, 83%/17% LN-/LN+ ILC, 95% ET-treated, 17% CT-treated IDC, 94% ET-treated, 25% CT-treated	5-year OS	p < 0.0001; HR for OS, RS 0–25 vs. RS 26–100–25 patients, HR, 0.44, 95% CI: 0.28–0.68; p = 0.00014 - ILC: OS, p = 0.87; DFS, p = 0.36 - IDC patients were more likely than ILC patients to have RS 26–100 (16% vs. 7%, p < 0.001)^c The RS was a significant prognosticator for ILC^d; - 5-year OS rates for RS 0–10, 11–25, and 26–100 patients, were 96.7%, 96.8%, and 93.0%, respectively (p < 0.0001). The difference remained significant in an MVA. CT benefit in ILC patients^d; - Adjuvant CT was significantly associated with OS benefit in RS 26–100 patients, particularly if they had LN+ disease.

Abbreviations: BC, breast cancer; BCCS, breast cancer-specific survival; CI, confidence interval; CT, chemotherapy; DMFI, distant-metastasis free interval; DMFS, distant-metastasis-free survival; DR, distant recurrence; DRFS, distant recurrence-free survival; ER, estrogen receptor; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LN, lymph node; MVA, multivariate analysis; NCDB, National Cancer Database; OS, overall survival; RS, Recurrence Score; SEER, the Surveillance, Epidemiology, and End Results program.

^aAs reported, not all articles provided the same level of detail.

^bER+ subset for which clinical outcome analyses were performed.

^cComparison of the RS distributions.

^dThe report did not provide the data for IDC.

TABLE 3 Summary of cross-platform comparison studies evaluating discordance/correlations between GEAs performed on the same tumor samples.

Author(s)/ # reference(s)	Study design, N	Assays compared	Patient population	Main results
1 Varga et al. ¹²⁶	Summary of 14 studies with cross-platform comparisons, of which 10 included discordance data between Oncotype DX and other GEAs. Discordance was categorized as minor (a single risk-group difference) or major (more than a single risk-group difference).	Oncotype DX vs. BCI, EndoPredict (EP/EPclin), MammaPrint, and Prosigna	The studies included were mostly retrospective, except for TransATAC and OPTIMA, which are described below.	Oncotype DX vs. BCI (1 study): -Minor discordances, 37% of cases; major discordances 5% of cases. Oncotype DX vs. EP/EPclin (1 study): -Minor discordances, 29%/29% of cases; major discordances 18%/21% of cases. Oncotype DX vs. MammaPrint (4 studies): -Minor discordances, 26%–38% of cases; major discordances, 19%–25% of cases. Oncotype DX vs. Prosigna (4 studies): -Minor discordances, 37%–45% of cases; major discordances 3%–20% of cases. - The proportions of patients classified as high-risk varied according to the assay. In a pooled analysis of all 10 studies (n = 5514) these proportions were: Oncotype DX, 11.5%; BCI, 16.2%; Prosigna, 30.7%; MammaPrint, 45.8%; EPclin, 48.4%; and EP 63.0%.
2 Stein et al. ¹²⁷ ; Bartlett et al. ¹²⁸	Analysis of the OPTIMA prelim study, a feasibility phase of a prospective randomized trial, which evaluated the use of GEAs in selecting treatment compared to standard treatment (CT followed by ET).	MammaPrint, Oncotype DX Prosigna ^a	302 tumor samples All from BC patients ≥40 years old, with ER+ HER2– disease who were LN+ or had tumor ≥30 mm	MammaPrint vs. Oncotype DX (n = 297): -Discordance (low/intermediate ^b vs. high): 25.6% MammaPrint vs. Prosigna (n = 295): -Discordance (low/intermediate ^b vs. high): 21.7% Oncotype DX vs. Prosigna (n = 298): -Discordance (low/intermediate vs. high): 22.5% The proportions of patients classified as high-risk varied according to the assay: Oncotype DX (n = 301), 17.9%; Prosigna (n = 299), 34.5%; MammaPrint (n = 298), 38.6%.
3 Buus et al. ¹¹⁶	Analysis of the transATAC study, a prospective randomized trial, which compared anastrozole to TAM in postmenopausal patients (all cDNA was produced by GHI and provided to the transATAC investigators). The study evaluated the GEA scores as continuous variables. Only the molecular components of the BCI, EP and Prosigna ROR were assessed.	BCI, EndoPredict, Oncotype DX, Prosigna	785 tumor samples All patients were HR+, HER2–, CT-untreated, and samples had to have scores for all 4 evaluated assays.	In pairwise comparisons, the correlations were variable and poorer for Oncotype DX against most competing assays: - Prosigna vs. BCI, $\rho = 0.74$ - Prosigna vs. EndoPredict, $\rho = 0.68$ - EndoPredict vs. BCI, $\rho = 0.67$ - Oncotype DX vs. EndoPredict, $\rho = 0.63$ - Oncotype DX vs. BCI, $\rho = 0.35$ - Oncotype DX vs. Prosigna, $\rho = 0.32$

Abbreviations: BC, breast cancer; BCI, Breast Cancer Index; CT, chemotherapy; ER, estrogen receptor; ET, endocrine therapy; GEA, gene expression assay; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; LN, lymph node; RS, Recurrence Score; TAM, tamoxifen.
^aIHC and IHC4-AQUA which are not discussed in the current review were also analyzed in OPTIMA Prelim.
^bFor the RS, low/intermediate range was defined as RS 0–25.

TABLE 4 Summary of cross-platform analyses evaluating prognostic output using the transATAC data.

Author(s)/ # reference(s)	Objective, primary endpoint, N	Assays compared	Patient population	Main results
1 Sgroi et al. ²³	To evaluate the ability of GEAs to predict recurrence beyond standard clinicopathologic parameters. Primary endpoint: DR n = 665	BCI ^a and Oncotype DX	Postmenopausal, ER+, LN- ET-treated (anastrozole or TAM for 5 years), CT-untreated	Multivariate analysis (including CTS) in all ER+ LN- patients (HR values are for a change between the 25 th and 75 th percentile). DR risk, years 0–5 -BCI: HR, 2.8 (95% CI: 1.6–4.7); LR-Δχ ² = 15.4; p < 0.0001 -Oncotype DX: HR, 1.8 (95% CI, 1.4–2.3); LR-Δχ ² = 18.5, p < 0.0001 DR risk, years 5–10 -BCI: HR, 1.95 (95% CI: 1.2–3.1); LR-Δχ ² = 8.0; p = 0.0048 -Oncotype DX: HR, 1.1 (95% CI: 0.82–1.6); LR-Δχ ² = 0.48, p = 0.47
2 Sestak et al. ¹²⁹	To evaluate the ability of GEAs to predict recurrence beyond standard clinicopathologic parameters, upon stratifying patients by age and BMI. Primary endpoint: DR n = 940 (analysis by age) n = 865 (analysis by BMI)	Oncotype DX, and Prosigna ^b	Postmenopausal, hormone receptor+, ET-treated (anastrozole or TAM for 5 years), CT-untreated	Bivariate analysis (in addition to CTS) for DR risk, years 0–10 (HR values are for a change between the 25 th and 75 th percentile). By age (split according to tertiles) -Oncotype DX: <59.8 years, HR, 1.8 (95% CI: 1.3–2.4); LR-Δχ ² = 13.3; 59.8–68.2 years, HR, 1.3 (95% CI: 1.04–1.6); LR-Δχ ² = 4.7; >68.2 years, HR, 1.3 (95% CI: 1.0–1.6); LR-Δχ ² = 3.6. -Prosigna: <59.8 years, HR, 2.1 (95% CI: 1.1–3.8); LR-Δχ ² = 5.5; 59.8–68.2 years, HR, 3.2 (95% CI: 2.0–5.2); LR-Δχ ² = 25.0; >68.2 years, HR, 1.3 (95% CI: 0.9–1.9); LR-Δχ ² = 2.3. By BMI (standard BMI categories) -Oncotype DX: <25 kg/m ² , HR, 1.5 (95% CI: 1.2–2.0); LR-Δχ ² = 9.0; 25–30 kg/m ² , HR, 1.4 (95% CI: 1.2–1.7); LR-Δχ ² = 10.8; >30 kg/m ² , HR, 1.1 (95% CI: 0.77–1.5); LR-Δχ ² = 0.15. -Prosigna: <25 kg/m ² , HR, 1.9 (95% CI: 1.2–3.1); LR-Δχ ² = 7.3; 25–30 kg/m ² , HR, 2.3 (95% CI: 1.6–3.5); LR-Δχ ² = 16.6; >30 kg/m ² , HR, 2.4 (95% CI: 1.2–4.7); LR-Δχ ² = 6.6.
3 Sestak et al. ¹¹⁵	To evaluate the ability of GEAs to predict recurrence beyond standard clinicopathologic parameters, upon stratifying patients by nodal status. Primary endpoint: DR n = 774	BCI, EndoPredict (EPclin), Oncotype DX, and Prosigna ^b	Postmenopausal, ER+, HER2-, LN-/LN+ (up to 3 positive nodes), ET-treated (anastrozole or TAM for 5 years), CT-untreated	Univariate analysis by nodal status for DR risk, years 0–10 (HRs per SD change) LN- (n = 591) BCI: HR, 2.5 (95% CI: 1.9–3.2); c-index, 0.76 (0.70–0.82)

TABLE 4 (Continued)

Author(s)/ # reference(s)	Objective, primary endpoint, N	Assays compared	Patient population	Main results
				EPclin: HR, 2.1 (95% CI: 1.7–2.7); c-index, 0.765 (0.72–0.81) Oncotype DX: HR, 1.7 (95% CI: 1.4–2.0); c-index, 0.67 (0.59–0.75) Prosigna: HR, 2.6 (95% CI: 2.0–3.4); c-index, 0.76 (0.71–0.82) LN+ (n = 227) BCI: HR, 1.7 (95% CI: 1.2–2.3); c-index, 0.65 (0.57–0.74) EPclin: HR, 1.7 (95% CI: 1.3–2.2); c-index, 0.67 (0.59–0.75) Oncotype DX: HR, 1.4 (95% CI: 1.05–1.9); c-index, 0.60 (0.51–0.69) Prosigna: HR, 1.6 (95% CI: 1.2–2.2); c-index, 0.64 (0.55–0.72)
4 Buus et al. ¹³⁰	To compare the prognostic utility of a novel signature to currently available GEAs. ^c Primary endpoint: DR n = 271 (transATAC validation cohort)	BCI, Oncotype DX, and Prosigna	Postmenopausal, ER+, HER2–, ET-treated (anastrozole or TAM for 5 years), CT-untreated, with data for all evaluated GEAs	Multivariate analysis (including CTS) for DR risk, years 0–10 (HRs per SD change). -BCI: HR, 2.0 (95% CI: 1.4–3.0); LR-Δχ ² = 14.5; p < 0.001 -Oncotype DX: HR, 1.4 (95% CI: 1.1–1.8); LR-Δχ ² = 5.1, p = 0.024 -Prosigna: HR, 1.6 (95% CI: 1.1–2.2); LR-Δχ ² = 7.5, p = 0.006

Abbreviations: BC, breast cancer; BCI, Breast Cancer Index; BMI, body mass index; CI, confidence interval; CTS, clinical treatment score; DR, distant recurrence; ER, estrogen receptor; GEA, gene expression assay; HR, hazard ratio; LN, lymph node; TAM, tamoxifen.
BCI-cubic and BCI-linear models were used in the analysis. The current table presents the commercially adopted BCI-linear model data.
IHC4 which is not discussed in the current review was also evaluated in this analysis.
Only data for the GEAs discussed in the current review are presented.

the prognostic value of an assay. Using this approach, BCI, Prosigna-ROR, and EPclin have been shown to provide the most independent prognostic information, followed by the RS result. And yet, the Oncotype DX assay has the most robust clinical trial data supporting its prognostic and predictive capabilities of these four GEAs. MammaPrint was not evaluated in this series of studies and could not be so evaluated.

4 | DISCUSSION

The development of gene profiling has enabled a major paradigm shift for women with ESBC, identifying a significant population of patients who may reasonably avoid CT. Five GEAs have been widely commercialized, including BCI, EndoPredict, MammaPrint, Oncotype DX, and Prosigna. Most were initially validated through “prospective-retrospective” studies, confirming their prognostic capabilities with high LOE. The Oncotype DX assay was demonstrated to predict CT value in individual patients.^{18,131} However, at the time of our 2017 review, only two, Oncotype DX and MammaPrint assays, had been subjected to large prospective clinical trials. The MammaPrint GEA was evaluated in MINDACT to assess a cH population of ESBC patients. The 10-year risk, when shown to be gL by MammaPrint, was sufficiently low to justify CT avoidance for the entire ITT cohort of cHgL patients.⁹ Updated results of MINDACT are consistent with the original analysis.⁴⁰ Yet the population for whom these findings are relevant represented only 23% of the MINDACT study population (i.e., no treatment guidance was provided for cLgH or cHgL patients). Furthermore, although the randomized CT treatment arms failed to demonstrate predictive value for CT benefit overall with only a non-significant 2.6% trend in CT-associated DMFS, subset analysis of patients ≤ 50 suggested that younger patients with cHgL had a clinically meaningful 5% absolute benefit of CT. As the assay was not useful in younger patients with cLgH, MammaPrint seems of little value for putatively premenopausal patients ≤ 50 .

The duration of use and the associated side effects of endocrine agents lead many patients to abandon ET, despite efficacy in reducing BC mortality during the first 15 years after diagnosis.¹³² Recent studies have suggested that only 74% of patients adhere to adjuvant ET.¹³³ The establishment of a new gUL-risk threshold for MammaPrint has identified a group of patients who might de-escalate ET. Although most of the gUL patients (15% of MINDACT) were LN- and/or had favorable clinicopathologic features, not all did. Unfortunately, as “no-treatment” was not a protocol-recognized management option in MINDACT, de-escalation could not be properly evaluated. Although 84% of gUL patients received adjuvant therapy, some did not. They had similar excellent outcomes as those treated with ET with 8-year DMFI rates $>97\%$.

Baseline risk was also assessed in the prospective phase III Oncotype DX trials. Both the WSG PlanB and TAILORx trials identified ESBC patients with very low risk. For PlanB, ET-treated patients with $RS \leq 11$ exceeded 94% DFS for the overall group, as well as for the N0 and N1 subgroups.⁷¹ In TAILORx, 5-year iDFS with

ET only was similar to that in PlanB at 94% for patients with $RS < 11$; DRFI was 99.3% at 5 years and 96.8% at 9 years.¹⁰ Consistent with the gUL-risk findings of MammaPrint, patients may now be identified whose risk is sufficiently low to consider ET de-escalation. Confirmation would require a very large randomized trial with a no ET treatment arm, which given the low event rate may not be a practical enterprise.

A primary endpoint for both TAILORx and RxPONDER was the prospective confirmation of Oncotype DX CT prediction. Both studies incorporated ET versus CT + ET randomizations, with TAILORx targeting RS 11–25 patients, and RxPONDER RS 0–25 patients. Both studies confirmed an absence of CT benefit in N0 or N1 disease when $RS \leq 25$ in the ITT population. However, in a subset analysis, a modest benefit was noted for LN- women ≤ 50 and RS 16–25, and for LN+ premenopausal women with RS 0–25.^{10,12}

In LN- women ≤ 50 , TAILORx investigators evaluated the RS result with a categorical clinical risk assessment based on tumor size and grade. The *post hoc* analysis suggested that only cH patients with RS 16–20 benefit from CT with an advantage of 6.5%, albeit with fairly wide CIs.⁷⁰ Nonetheless, for such women, use of the clinical risk assessment might be helpful for the subset of individuals considering CT.

That menopausal status and/or age were noted in both the TAILORx and RxPONDER trials suggests that this finding is not spurious. Indeed, Piccart’s MINDACT update also suggested that any CT benefit in the cHgL population was limited to premenopausal patients.⁴⁰ Investigators in all three trials wondered whether CT-induced ovarian function suppression (OFS) might be responsible for this finding. The SOFT trial, which evaluated the role of OFS with ET, demonstrated a similar magnitude of benefit in premenopausal patients.^{134,135} However, TAILORx and RxPONDER had insufficient data and relevant endpoint events to resolve this issue.^{10,12} The question of whether CT adds more than OFS will await further information from studies such as the ongoing NCI-sponsored OFSET trial (NRG-BR009).¹³⁶

Given the data for CT benefit, some suggest that the Oncotype DX assay be avoided in premenopausal LN+ patients. However, the observed benefit of CT was an overall 3.3% DRFS difference. As the impact of CT occurs during the first 5 years, further maturation of data is not likely to alter the magnitude of benefit.¹³⁷ This statistically significant advantage may not be clinically significant for all patients. The RS result, providing long-term DRFS estimates, may signify a sufficiently low risk for some women to bypass CT rather than pursue a small single-digit percentage-point improvement at the cost of near and long-term toxicity. Consideration of CT, using a personalized biological risk estimate, mirrors risk/benefit assessment of adjuvant ET for gUL individuals. GEA testing in both settings allows for better shared physician-patient decision-making. Although the Oncotype DX assay provides guidance regarding the use of CT, data from the WSG PlanB, ADAPT, NSABP B-28, and PACS01 trials indicate that it does not provide information for the preferential selection of one CT regimen over another.^{78,80}

Both B-20 and SWOG 8814 used a cut point of RS 31, with a $RS \geq 31$ indicating substantial benefit to CT use. TAILORx and

RxPONDER only randomized CT up to an RS result of 25. A re-analysis of NSABP B-20 was performed including patients with RS ≥ 26 while excluding HER2+ patients to mirror today's Oncotype DX target population. Although the HR benefit remained advantageous for CT (HR = 0.27), it was diminished by 0.09 suggesting a "dilution" effect by lowering the cut point.⁷⁸ CT effect between RS results of 25 and 30 may be present, but is likely less robust than for RS ≥ 31 patients. Unfortunately, a precise estimation of this benefit could not be identified. Nonetheless, data from SEER and NCDB suggest a BCSM improvement in patients receiving CT with RS 26–30.^{86,87}

Although CT effect is an early one, ET has impact over a much longer time frame, leading to interest in EET.¹³⁸ The value of EET with AIs, showing a more modest DFS improvement, and no OS advantage, makes the issue of residual risk and therapeutic benefit beyond 5 years a relevant one.¹³⁹ BCI was originally developed as a prognostic assay to evaluate risk from time of diagnosis. However, its commercial development has focused on an ability to distinguish late DR between 5 and 10 years. This was demonstrated in a direct comparison with the Oncotype DX assay in the ATAC trial samples, where only BCI provided prognostic information regarding risk after 5 years.²³ Efforts to better evaluate the Oncotype DX assay in this setting, through a re-analysis of NSABP B-14 and B-28 produced somewhat conflicting results.⁸² In the LN+ population of B-28, Wolmark⁸² and colleagues demonstrated prognostic value for the RS result in years 5–10. However, this was not evident in the lower-risk B-14 LN- population unless ESR1 expression was used to risk-stratify patients. Performing an additional GEA, such as BCI, to guide EET may identify the minority of patients who derive benefit from this added intervention, while increasing compliance with the EET regimen.¹⁴⁰

EndoPredict also has a discriminatory value in separating postmenopausal HR+, HER2-, T0-T1 patients into low- and high-risk populations. However, the GEA by itself was not as robust a prognostic until combined with nodal status and tumor size to calculate the clinically influenced EPclin score. Although EndoPredict has high LOE validation for prognosis, the population used in its studies limits its use to postmenopausal women. Premenopausal patients were evaluated in GEICAM 9906, but all received CT making baseline prognosis unavailable.^{5,31–33} Furthermore, there is no study that supports EPclin as a predictor of response or resistance to CT. Efforts at in silico modeling, such as those described by Soliman³⁶ and colleagues, require too many assumptions to compete with the prospective validation data of Oncotype DX.

Although EPclin has been suggested for EET decision-making, the available data are problematic. The GEA identified an 11% 10-year cumulative difference in risk group performance for LN- individuals, but the difference was front-loaded and appeared to disappear after 5 years of ET. There was little meaningful difference between the two groups in the time from 5 to 15 years.³⁴ This, coupled with increasing scrutiny of EET use overall, has largely sidelined EndoPredict for EET decision-making.

The Prosigna assay combines a scientifically derived GEA with a newer analytic tool that enables local specimen processing and evaluation. The hybridization technique, using the nCounter analytic

system, can be purchased or leased by local hospitals, health systems, or laboratory companies. However, the reagents and local skill set required may make the cost comparable to other GEAs. Prosigna produces ROR score as well as information regarding the intrinsic subtype of the tested tumor. Because of its validation trials, data had only been available for postmenopausal HR+ patients, and provided no information predictive of treatment. Jensen⁹⁸ and colleagues have tried to address these limitations in a study based on a 46-year-old Danish randomized clinical CT trial DBCG 77B with no significant CT advantage based on ROR or assignment to any of the subtypes except Her2-enriched. Additional studies using the Danish DBCG 89D evaluated the difference between adjuvant CMF versus CEF. Although no impact of ROR on the specific treatment regimen could be found, patients with tumors assigned to the Her2-enriched subtype had a disproportionate benefit from the anthracycline regimen.¹⁰⁰ Given the toxicity of anthracyclines, and prospective data available for anthracycline- versus taxane-based therapy for HER2 overexpressing patients, the relevance of this finding is uncertain.

Prosigna and the Oncotype DX assays have generated data suggesting that higher ROR or RS result are associated with increased risk of local recurrence. However, there is no data supporting either GEA as an indicator for more aggressive surgery or for the use of RT.

Beyond prognosis, however, only the Oncotype DX assay continues to have predictive value with regard to CT selection. Some have suggested that GEAs are largely equivalent and that the predictive value of the Oncotype DX assay would likely extend to the remaining assays. However, the data do not suggest concordance or equivalency between these five commercially available GEAs. Most of the comparisons have not been direct and were not performed by the relevant commercial laboratories in a real-world setting. The transATAC population analyses are the only studies with long-term follow-up directly comparing GEAs using mRNA from the same patient specimens.^{23,115,129,130} These comparisons assessed the prognostic value of each assay using likelihood ratios and the c-index. However, these analyses are limited to postmenopausal women who received ET. They are likely biased toward lower-risk patients and do not address prediction of CT benefit. In transATAC, patients were selected for CT based on clinicopathological characteristics resulting in likely selection bias across the study cohort. Because the IHC4 and the cut points for the ROR were trained in the transATAC cohort, there is increased risk of overfitting, and without independent validation cohort data available, it is difficult to confirm the degree of model overfitting present. In evaluating the use of likelihood ratio and c-index statistics, numerical comparisons of prognostic ability are not indicative of statistical independence. In addition, an apparent increase in model fit across survival models using likelihood ratio tests is less intuitive than a comparison of fit using nested models. Consequently, clinical choice of a GEA should be made with the target population in mind and based upon the LOE of the data available to support the GEA use in that population, rather than by statistical comparisons.

GEAs have markedly changed the risk assessment of ESBC. Having an accurate estimate of early and late recurrence risk can

change the calculus of immediate and delayed post-operative adjuvant therapy decision-making. If risk is sufficiently low, the issue of drug efficacy becomes less relevant as even low or moderate toxicities may obviate a slight statistical benefit to any given treatment. However, as risk increases, having a clear and unambiguous appreciation for the value of adjuvant therapies is important in guiding decision-making. In this setting, a GEA with proven ability to predict therapeutic benefit, confirmed in a prospective clinical trial, becomes the genomic test of choice.

5 | CONCLUSIONS

The use of GEAs in ESBC has been practice changing. The five widely commercialized assays all have some high-level evidence supporting prognostic capability in varying patient subsets. Several of the GEAs identify low-risk patients for whom adjuvant CT is difficult to justify. Two assays (MammaPrint and Oncotype DX) identify patients who might reasonably avoid even ET, although current data remain preliminary. Some assays provide late prognostic information that may influence selection of EET. BCI, the most widely used in this setting, also appears predictive of response to anti-estrogens. Nonetheless, several GEAs remain unvalidated in certain subpopulations of patients based on menopausal and lymph node status. Only two assays (MammaPrint and Oncotype DX) have been subjected to large prospective clinical trials. Only the Oncotype DX assay has high LOE support for predicting adjuvant CT benefit. That two of three prospective trials of the Oncotype DX assay identified a relationship between CT benefit and age and/or menopause, highlights the importance of large trials in refining care. The wealth of new data generated for the five widely commercialized GEAs provides a basis for thoughtful assessment of each assay, and its ability to provide clinically relevant prognostic and predictive information in ESBC.

ACKNOWLEDGMENTS

Assistance with biostatistical interpretation was kindly provided by Jess Hoag, PhD, MPH. No direct financial support or grant has been provided for the conduct of this review.

CONFLICT OF INTEREST STATEMENT

All authors completed a disclosure declaration. The following authors indicated a financial or other interest that is relevant to the subject matter under consideration in this article: Employment or leadership position: None. Stock ownership: None. Consultant, advisor, or speaker: David M. Hyams, Exact Sciences, Castle Biosciences; Avital Bareket-Samish, Exact Sciences, Rhenium-Oncotest; Juan Enrique Bargallo Rocha, Roche, Exact Sciences; Sandra Franco, Roche, Merck; Debora Gagliato, Roche, Daiichi Sankyo/Lilly; Henry L. Gomez, Roche, Exact Sciences; Andre Mattar, Fleury, Roche; Anibal Nunez De Pierro, Exact Sciences, Omics SRL, South Genetics; Manuel Ruiz Borrego, Daiichi.

DATA AVAILABILITY STATEMENT

Data sharing not applicable.

ORCID

David M. Hyams  <http://orcid.org/0000-0002-5680-9488>

REFERENCES

1. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med*. 2002;347(25):1999-2009.
2. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351(27):2817-2826.
3. Gnant M, Filipits M, Greil R, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinico-pathological risk: using the PAM50 risk of recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol*. 2014;25(2):339-345.
4. Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol*. 2013;31(22):2783-2790.
5. Filipits M, Rudas M, Jakesz R, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res*. 2011;17(18):6012-6020.
6. Cuzick J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the genomic health recurrence score in early breast cancer. *J Clin Oncol*. 2011;29(32):4273-4278.
7. Ma XJ, Salunga R, Dahiya S, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. *Clin Cancer Res*. 2008;14(9):2601-2608.
8. Ma XJ, Wang Z, Ryan PD, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell*. 2004;5(6):607-616.
9. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med*. 2016;375(8):717-729.
10. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med*. 2018;379(2):111-121.
11. Sparano JA, Gray RJ, Makower DF, et al. Prospective validation of a 21-gene expression assay in breast cancer. *N Engl J Med*. 2015;373(21):2005-2014.
12. Kalinsky K, Barlow WE, Gralow JR, et al. 21-gene assay to inform chemotherapy benefit in node-positive breast cancer. *N Engl J Med*. 2021;385(25):2336-2347.
13. Hyams DM, Schuur E, Angel Aristizabal J, et al. Selecting postoperative adjuvant systemic therapy for early stage breast cancer: a critical assessment of commercially available gene expression assays. *J Surg Oncol*. 2017;115(6):647-662.
14. Griguolo G, Bottosso M, Vernaci G, Miglietta F, Dieci MV, Guarneri V. Gene-expression signatures to inform neoadjuvant treatment decision in HR+/HER2- breast cancer: available evidence and clinical implications. *Cancer Treat Rev*. 2022;102:102323.
15. Wolff AC, Berry D, Carey LA, et al. Research issues affecting preoperative systemic therapy for operable breast cancer. *J Clin Oncol*. 2008;26(5):806-813.

16. Zhang Y, Schnabel CA, Schroeder BE, et al. Breast Cancer Index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. *Clin Cancer Res*. 2013;19(15):4196-4205.
17. Bartlett JMS, Sgroi DC, Treuner K, et al. Breast Cancer Index and prediction of benefit from extended endocrine therapy in breast cancer patients treated in the adjuvant Tamoxifen—to offer more? (aTTom) trial. *Ann Oncol*. 2019;30(11):1776-1783.
18. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst*. 2009;101(21):1446-1452.
19. Ma XJ, Hilsenbeck SG, Wang W, et al. The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer. *J Clin Oncol*. 2006;24(28):4611-4619.
20. Goetz MP, Suman VJ, Ingle JN, et al. A two-gene expression ratio of homeobox 13 and interleukin-17B receptor for prediction of recurrence and survival in women receiving adjuvant tamoxifen. *Clin Cancer Res*. 2006;12(7 Pt 1):2080-2087.
21. Ma XJ, Salunga R, Tuggle JT, et al. Gene expression profiles of human breast cancer progression. *Proc Natl Acad Sci USA*. 2003;100(10):5974-5979.
22. Schroeder B, Zhang Y, Stål O, et al. Risk stratification with Breast Cancer Index for late distant recurrence in patients with clinically low-risk (T1N0) estrogen receptor-positive breast cancer. *NPJ Breast Cancer*. 2017;3:28.
23. Sgroi DC, Sestak I, Cuzick J, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol*. 2013;14(11):1067-1076.
24. Zhang Y, Schroeder BE, Jerevall PL, et al. A novel Breast Cancer Index for prediction of distant recurrence in HR(+) early-stage breast cancer with one to three positive nodes. *Clin Cancer Res*. 2017;23(23):7217-7224.
25. Jansen MPH, Sieuwerts AM, Look MP, et al. HOXB13-to-IL17BR expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study. *J Clin Oncol*. 2007;25(6):662-668.
26. Jerevall PL, Brommesson S, Strand C, et al. Exploring the two-gene ratio in breast cancer--independent roles for HOXB13 and IL17BR in prediction of clinical outcome. *Breast Cancer Res Treat*. 2008;107(2):225-234.
27. Sgroi DC, Carney E, Zarrella E, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. *J Natl Cancer Inst*. 2013;105(14):1036-1042.
28. Noordhoek I, Treuner K, Putter H, et al. Breast Cancer Index predicts extended endocrine benefit to individualize selection of patients with HR(+) early-stage breast cancer for 10 years of endocrine therapy. *Clin Cancer Res*. 2021;27(1):311-319.
29. Bartlett JMS, Sgroi DC, Treuner K, et al. Breast Cancer Index is a predictive biomarker of treatment benefit and outcome from extended tamoxifen therapy: final analysis of the trans-atom study. *Clin Cancer Res*. 2022;28(9):1871-1880.
30. Brase J, Kronenwett R, Petry C, Denkert C, Schmidt M. From high-throughput microarray-based screening to clinical application: the development of a second generation multigene test for breast cancer prognosis. *Microarrays*. 2013;2(3):243-264.
31. Martin M, Brase JC, Calvo L, et al. Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2- breast cancer patients: results from the GEICAM 9906 trial. *Breast Cancer Res*. 2014;16(2):R38.
32. Dubsky P, Filipits M, Jakesz R, et al. EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer. *Ann Oncol*. 2013;24(3):640-647.
33. Buus R, Sestak I, Kronenwett R, et al. Comparison of EndoPredict and EPclin with Oncotype DX recurrence score for prediction of risk of distant recurrence after endocrine therapy. *J Natl Cancer Inst*. 2016;108(11):djw149.
34. Filipits M, Dubsky P, Rudas M, et al. Prediction of distant recurrence using EndoPredict among women with ER(+), HER2(-) node-positive and node-negative breast cancer treated with endocrine therapy only. *Clin Cancer Res*. 2019;25(13):3865-3872.
35. Ribnikar D, Sousa B, Cufer T, Cardoso F. Extended adjuvant endocrine therapy—a standard to all or some? *The Breast*. 2017;32:112-118.
36. Soliman H, Flake 2nd DD, Magliocco A, et al. Predicting expected absolute chemotherapy treatment benefit in women with early-stage breast cancer using EndoPredict, an integrated 12-gene clinicomolecular assay. *JCO Precis Oncol*. 2019;3:1-10.
37. Sestak I, Martín M, Dubsky P, et al. Prediction of chemotherapy benefit by EndoPredict in patients with breast cancer who received adjuvant endocrine therapy plus chemotherapy or endocrine therapy alone. *Breast Cancer Res Treat*. 2019;176(2):377-386.
38. van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. 2002;415(6871):530-536.
39. Buyse M, Loi S, van't Veer L, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst*. 2006;98(17):1183-1192.
40. Piccart M, van't Veer LJ, Poncet C, et al. 70-gene signature as an aid for treatment decisions in early breast cancer: updated results of the phase 3 randomised MINDACT trial with an exploratory analysis by age. *Lancet Oncol*. 2021;22(4):476-488.
41. Aalders KC, Kuijter A, Straver ME, et al. Characterisation of multifocal breast cancer using the 70-gene signature in clinical low-risk patients enrolled in the EORTC 10041/BIG 03-04 MINDACT trial. *Eur J Cancer*. 2017;79:98-105.
42. Delahaye LJM, Drukker CA, Dreezen C, et al. A breast cancer gene signature for indolent disease. *Breast Cancer Res Treat*. 2017;164(2):461-466.
43. Esserman LJ, Yau C, Thompson CK, et al. Use of molecular tools to identify patients with indolent breast cancers with ultralow risk over 2 decades. *JAMA Oncol*. 2017;3(11):1503-1510.
44. Lopes Cardozo JMN, Drukker CA, Rutgers EJT, et al. Outcome of patients with an ultralow-risk 70-gene signature in the MINDACT trial. *J Clin Oncol*. 2022;40(12):1335-1345.
45. Opdam M, van der Noort V, Kleijn M, et al. Limiting systemic endocrine overtreatment in postmenopausal breast cancer patients with an ultralow classification of the 70-gene signature. *Breast Cancer Res Treat*. 2022;194(2):265-278.
46. Noordhoek I, Bastiaannet E, de Glas NA, et al. Validation of the 70-gene signature test (MammaPrint) to identify patients with breast cancer aged ≥ 70 years with ultralow risk of distant recurrence: a population-based cohort study. *J Geriatr Oncol*. 2022;13(8):1172-1177.
47. Vliek SB, Hilbers FS, Jager A, et al. Ten-year follow-up of the observational RASTER study, prospective evaluation of the 70-gene signature in ER-positive, HER2-negative, node-negative, early breast cancer. *Eur J Cancer*. 2022;175:169-179.
48. Dubsky P, Van't Veer L, Gnant M, et al. A clinical validation study of MammaPrint in hormone receptor-positive breast cancer from the Austrian breast and colorectal cancer study group 8 (ABCSG-8) biomarker cohort. *ESMO Open*. 2021;6(1):100006.
49. Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). *Lancet Oncol*. 2007;8(12):1079-1087.
50. Gluz O, Nitz UA, Christgen M, et al. West German Study Group Phase III PlanB Trial: first prospective outcome data for

the 21-gene recurrence score assay and concordance of prognostic markers by central and local pathology assessment. *J Clin Oncol*. 2016;34(20):2341-2349.

51. Markopoulos C, van de Velde C, Zarca D, Ozmen V, Masetti R. Clinical evidence supporting genomic tests in early breast cancer: do all genomic tests provide the same information? *Eur J Surg Oncol*. 2017;43(5):909-920.
52. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010;11(1):55-65.
53. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006;24(23):3726-3734.
54. Kuchel A, Robinson T, Comins C, et al. The impact of the 21-gene assay on adjuvant treatment decisions in oestrogen receptor-positive early breast cancer: a prospective study. *Br J Cancer*. 2016;114(7):731-736.
55. Albanell J, Svedman C, Gligorov J, et al. Pooled analysis of prospective European studies assessing the impact of using the 21-gene recurrence score assay on clinical decision making in women with oestrogen receptor-positive, human epidermal growth factor receptor 2-negative early-stage breast cancer. *Eur J Cancer*. 2016;66:104-113.
56. Ozmen V, Atasoy A, Gokmen E, et al. Impact of Oncotype DX recurrence score on treatment decisions: results of a prospective multicenter study in Turkey. *Cureus*. 2016;8(3):e522.
57. Zeng Y, Li Q, Qin T, et al. Impact of a 21-gene recurrence score test on the choice of adjuvant chemotherapy for hormone receptor-positive early-stage breast cancer: a prospective study. *Anticancer Res*. 2017;37(8):4539-4547.
58. Dieci MV, Guarneri V, Zustovich F, et al. Impact of 21-gene breast cancer assay on treatment decision for patients with T1-T3, NO-N1, estrogen receptor-positive/human epidermal growth receptor 2-negative breast cancer: final results of the prospective multicenter ROXANE study. *Oncologist*. 2019;24(11):1424-1431.
59. Hassan S, Younan R, Patocskai E, et al. Impact of the 21-gene recurrence score assay on treatment decisions and cost in patients with node-positive breast cancer: a multicenter study in Quebec. *Oncologist*. 2022;27(10):822-831.
60. Leung RCY, Yau TCC, Chan MCM, et al. The impact of the Oncotype DX breast cancer assay on treatment decisions for women with estrogen receptor-positive, node-negative breast carcinoma in Hong Kong. *Clin Breast Cancer*. 2016;16(5):372-378.
61. LeVasseur N, Sun J, Fenton D, et al. Impact of the 21-gene recurrence score assay on the treatment of estrogen receptor-positive, her2-negative, breast cancer patients with 1-3 positive nodes: a prospective clinical utility study. *Clin Breast Cancer*. 2022;22(1):e74-e79.
62. Levine MN, Julian JA, Bedard PL, et al. Prospective evaluation of the 21-gene recurrence score assay for breast cancer decision-making in Ontario. *J Clin Oncol*. 2016;34(10):1065-1071.
63. Mattar A, Fonseca GR, Romão MBA, et al. Substantial reduction in adjuvant chemotherapy with the use of the 21-gene test to manage early breast cancer in a public hospital in Brazil. *JCO Glob Oncol*. 2021;7:1003-1011.
64. Pestalozzi BC, Tausch C, Dedes KJ, et al. Adjuvant treatment recommendations for patients with ER-positive/HER2-negative early breast cancer by Swiss tumor boards using the 21-gene recurrence score (SAKK 26/10). *BMC Cancer*. 2017;17(1):265.
65. Torres S, Trudeau M, Gandhi S, et al. Prospective evaluation of the impact of the 21-gene recurrence score assay on adjuvant treatment decisions for women with node-positive breast cancer in Ontario, Canada. *Oncologist*. 2018;23(7):768-775.
66. Zambelli A, Simoncini E, Giordano M, et al. Prospective observational study on the impact of the 21-gene assay on treatment decisions and resources optimization in breast cancer patients in Lombardy: the BONDx study. *The Breast*. 2020;52:1-7.
67. Gomez HL, Bargallo-Rocha JE, Billingham RJ, et al. Practice-changing use of the 21-gene test for the management of patients with early-stage breast cancer in Latin America. *JCO Glob Oncol*. 2021;7:1364-1373.
68. Cognetti F, Masetti R, Fabi A, et al. PONDx: real-life utilization and decision impact of the 21-gene assay on clinical practice in Italy. *NPJ Breast Cancer*. 2021;7(1):47.
69. Augustovski F, Soto N, Caporale J, Gonzalez L, Gibbons L, Ciapponi A. Decision-making impact on adjuvant chemotherapy allocation in early node-negative breast cancer with a 21-gene assay: systematic review and meta-analysis. *Breast Cancer Res Treat*. 2015;152(3):611-625.
70. Sparano JA, Gray RJ, Ravdin PM, et al. Clinical and genomic risk to guide the use of adjuvant therapy for breast cancer. *N Engl J Med*. 2019;380(25):2395-2405.
71. Nitz U, Gluz O, Christgen M, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. *Breast Cancer Res Treat*. 2017;165(3):573-583.
72. Nitz U, Gluz O, Christgen M, et al. Correction to: reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. *Breast Cancer Res Treat*. 2019;175(1):265-266.
73. Nitz UA, Gluz O, Kümmel S, et al. Endocrine therapy response and 21-gene expression assay for therapy guidance in HR+/HER2-early breast cancer. *J Clin Oncol*. 2022;40(23):2557-2567.
74. Sparano J, Gray RJ, Makower D, et al. Trial Assigning Individualized Options for Treatment (TAILORx): An Update Including 12-Year Event Rates [abstract]. In: Proceedings of the 2022 San Antonio Breast Cancer Symposium; 2022 Dec 6-10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2023;83(5 Suppl):Abstract nr GS1-05.
75. Kalinsky KM, Barlow WE, Gralow JR, et al. Updated results from a phase 3 randomized clinical trial in participants (pts) with 1-3 positive lymph nodes (LN), hormone receptor-positive (HR+) and HER2-negative (HER2-) breast cancer (BC) with recurrence score (RS)<25 randomized to endocrine therapy (ET) +/- chemotherapy (CT): SWOG S1007 (RxPONDER). Presented at SABCS; December 7-10, 2021; San Antonio, TX, USA.
76. Nitz U, Gluz O, Clemens M, et al. West German Study PlanB trial: adjuvant four cycles of epirubicin and cyclophosphamide plus docetaxel versus six cycles of docetaxel and cyclophosphamide in her2-negative early breast cancer. *J Clin Oncol*. 2019;37(10):799-808.
77. Nitz U, Gluz O, Kreipe HH, et al. The run-in phase of the prospective WSG-ADAPT HR+/HER2- trial demonstrates the feasibility of a study design combining static and dynamic biomarker assessments for individualized therapy in early breast cancer. *Ther Adv Med Oncol*. 2020;12:175883592097313.
78. Geyer Jr. CE, Tang G, Mamounas EP, et al. 21-Gene assay as predictor of chemotherapy benefit in HER2-negative breast cancer. *NPJ Breast Cancer*. 2018;4:37.
79. Mamounas EP, Tang G, Paik S, et al. 21-gene recurrence score for prognosis and prediction of taxane benefit after adjuvant chemotherapy plus endocrine therapy: results from NSABP B-28/NRG oncology. *Breast Cancer Res Treat*. 2018;168(1):69-77.
80. Mamounas EP, Liu Q, Paik S, et al. 21-gene recurrence score and locoregional recurrence in node-positive/ER-positive breast cancer treated with chemo-endocrine therapy. *J Natl Cancer Inst*. 2017; 109(4):djw259.

81. Penault-Llorca F, Filleron T, Asselain B, et al. The 21-gene Recurrence Score[®] assay predicts distant recurrence in lymph node-positive, hormone receptor-positive, breast cancer patients treated with adjuvant sequential epirubicin- and docetaxel-based or epirubicin-based chemotherapy (PACS-01 trial). *BMC Cancer*. 2018;18(1):526.
82. Wolmark N, Mamounas EP, Baehner FL, et al. Prognostic impact of the combination of recurrence score and quantitative estrogen receptor expression (ESR1) on predicting late distant recurrence risk in estrogen receptor-positive breast cancer after 5 years of tamoxifen: results from NRG Oncology/National surgical adjuvant breast and bowel project B-28 and B-14. *J Clin Oncol*. 2016;34(20):2350-2358.
83. Sparano JA, Cragger MR, Tang G, Gray RJ, Stemmer SM, Shak S. Development and validation of a tool integrating the 21-gene recurrence score and clinical-pathological features to individualize prognosis and prediction of chemotherapy benefit in early breast cancer. *J Clin Oncol*. 2021;39(6):557-564.
84. Petkov VI, Miller DP, Howlader N, et al. Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *NPJ Breast Cancer*. 2016;2:16017.
85. Park S, Han Y, Liu Y, et al. Adjuvant chemotherapy and survival among patients 70 years of age and younger with node-negative breast cancer and the 21-gene recurrence score of 26-30. *Breast Cancer Res*. 2019;21(1):110.
86. Ma SJ, Oladeru OT, Farrugia M, et al. Survival outcome of adjuvant chemotherapy and high 21-gene recurrence score in early-stage breast cancer. *Breast J*. 2021;27(1):27-34.
87. Yang SP, Yao J, Zhou P, et al. Adjuvant chemotherapy and survival outcome in node-negative breast cancer with a 21-gene recurrence score of 26-30. *Future Oncol*. 2021;17(17):2183-2192.
88. Ibraheem AF, Press DJ, Olopade OI, Huo D. Community clinical practice patterns and mortality in patients with intermediate Oncotype DX recurrence scores: who benefits from chemotherapy? *Cancer*. 2019;125(2):213-222.
89. Li GQ, Yao J, Zhou P, et al. Distribution, chemotherapy use, and outcome of the 21-gene recurrence score between Chinese and White breast cancer in the United States. *Clin Breast Cancer*. 2022;22(3):279-287.
90. Jung J, Hwang KT, Choi IS, et al. Racial differences in predictive value of the 21-gene recurrence score assay: a population-based study using the SEER database. *Breast Cancer*. 2022;29(5):889-898.
91. Gulbahce HE, White S, Herget KA, et al. 21-Gene recurrence score testing utilization among older women from different races: a population-based study. *J Geriatr Oncol*. 2021;12(2):206-211.
92. Hoskins KF, Danciu OC, Ko NY, Calip GS. Association of race/ethnicity and the 21-gene recurrence score with breast cancer-specific mortality among US women. *JAMA Oncol*. 2021;7(3):370-378.
93. Han Y, Miao ZF, Lian M, Peterson LL, Colditz GA, Liu Y. Racial and ethnic disparities in 21-gene recurrence scores, chemotherapy, and survival among women with hormone receptor-positive, node-negative breast cancer. *Breast Cancer Res Treat*. 2020;184(3):915-925.
94. Moore J, Wang F, Pal T, et al. Oncotype DX risk recurrence score and total mortality for early-stage breast cancer by race/ethnicity. *Cancer Epidemiol Biomarkers Prev*. 2022;31(4):821-830.
95. Albain KS, Gray RJ, Makower DF, et al. Race, ethnicity, and clinical outcomes in hormone receptor-positive, HER2-negative, node-negative breast cancer in the randomized TAILORx trial. *J Natl Cancer Inst*. 2021;113(4):390-399.
96. Perou CM, Sørli E, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
97. Nielsen T, Wallden B, Schaper C, et al. Analytical validation of the PAM50-based prosigna breast cancer prognostic gene signature assay and nCounter analysis system using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer*. 2014;14:177.
98. Jensen MB, Lænkholm AV, Nielsen TO, et al. The Prosigna gene expression assay and responsiveness to adjuvant cyclophosphamide-based chemotherapy in premenopausal high-risk patients with breast cancer. *Breast Cancer Res*. 2018;20(1):79.
99. Ejlersen B, Mouridsen HT, Jensen MB, et al. Cyclophosphamide, methotrexate, and fluorouracil; oral cyclophosphamide; levamisole; or no adjuvant therapy for patients with high-risk, premenopausal breast cancer. *Cancer*. 2010;116(9):2081-2089.
100. Jensen MB, Lænkholm AV, Balslev E, et al. The Prosigna 50-gene profile and responsiveness to adjuvant anthracycline-based chemotherapy in high-risk breast cancer patients. *NPJ Breast Cancer*. 2020;6:7.
101. Fitzal F, Filipits M, Fesl C, et al. PAM-50 predicts local recurrence after breast cancer surgery in postmenopausal patients with ER +/HER2- disease: results from 1204 patients in the randomized ABCSG-8 trial. *Br J Surg*. 2021;108(3):308-314.
102. Pramod N, Nigam A, Basree M, et al. Comprehensive review of molecular mechanisms and clinical features of invasive lobular cancer. *Oncologist*. 2021;26(6):e943-e953.
103. Nunes R, Sella T, Treuner K, et al. Prognostic utility of Breast Cancer Index to stratify distant recurrence risk in invasive lobular carcinoma. *Clin Cancer Res*. 2021;27(20):5688-5696.
104. Sestak I, Filipits M, Buus R, et al. Prognostic value of EndoPredict in women with hormone receptor-positive, HER2-negative invasive lobular breast cancer. *Clin Cancer Res*. 2020;26(17):4682-4687.
105. Beumer IJ, Persoon M, Witteveen A, et al. Prognostic value of MammaPrint[®] in invasive lobular breast cancer. *Biomarker Insights*. 2016;11:BMI.S38435.
106. Abel MK, Shui AM, Melisko M, et al. The incidence of discordant clinical and genomic risk in patients with invasive lobular or ductal carcinoma of the breast: a National Cancer Database Study. *NPJ Breast Cancer*. 2021;7(1):156.
107. Jenkins JA, Marmor S, Hui JYC, et al. The 70-gene signature test as a prognostic and predictive biomarker in patients with invasive lobular breast cancer. *Breast Cancer Res Treat*. 2022;191(2):401-407.
108. Kizy S, Huang JL, Marmor S, Tuttle TM, Hui JYC. Impact of the 21-gene recurrence score on outcome in patients with invasive lobular carcinoma of the breast. *Breast Cancer Res Treat*. 2017;165(3):757-763.
109. Turashvili G, Brogi E, Morrow M, et al. The 21-gene recurrence score in special histologic subtypes of breast cancer with favorable prognosis. *Breast Cancer Res Treat*. 2017;165(1):65-76.
110. Chen XH, Zhang WW, Wang J, et al. 21-gene recurrence score and adjuvant chemotherapy decisions in patients with invasive lobular breast cancer. *Biomark Med*. 2019;13(2):83-93.
111. Makower D, Qin J, Lin J, Xue X, Sparano JA. The 21-gene recurrence score in early non-ductal breast cancer: a National Cancer Database analysis. *NPJ Breast Cancer*. 2022;8(1):4.
112. Oesterreich S, Nasrazadani A, Zou J, et al. Clinicopathological features and outcomes comparing patients with invasive ductal and lobular breast cancer. *J Natl Cancer Inst*. 2022;114(11):1511-1522.
113. Weiser R, Polychronopoulou E, Hatch SS, et al. Adjuvant chemotherapy in patients with invasive lobular carcinoma and use of the 21-gene recurrence score: a National Cancer Database analysis. *Cancer*. 2022;128(9):1738-1747.
114. Sestak I, Zhang Y, Schroeder BE, et al. Cross-stratification and differential risk by Breast Cancer Index and recurrence score in women with hormone receptor-positive lymph node-negative early-stage breast cancer. *Clin Cancer Res*. 2016;22(20):5043-5048.
115. Sestak I, Buus R, Cuzick J, et al. Comparison of the performance of 6 prognostic signatures for estrogen receptor-positive breast cancer: a secondary analysis of a randomized clinical trial. *JAMA Oncol*. 2018;4(4):545-553.

116. Buus R, Sestak I, Kronenwett R, et al. Molecular drivers of Oncotype DX, Prosigna, EndoPredict, and the Breast Cancer Index: a TransATAC study. *J Clin Oncol*. 2021;39(2):126-135.
117. Gyanchandani R, Lin Y, Lin HM, et al. Intratumor heterogeneity affects gene expression profile test prognostic risk stratification in early breast cancer. *Clin Cancer Res*. 2016;22(21):5362-5369.
118. Bayani J, Poncet C, Crozier C, et al. Evaluation of multiple transcriptomic gene risk signatures in male breast cancer. *NPJ Breast Cancer*. 2021;7(1):98.
119. Bartlett JMS, Bayani J, Kornaga E, et al. Comparative survival analysis of multiparametric tests-when molecular tests disagree—A TEAM pathology study. *NPJ Breast Cancer*. 2021;7(1):90.
120. Buus R, Szigyarto Z, Schuster EF, et al. Development and validation for research assessment of Oncotype DX(R) breast recurrence score, EndoPredict(R) and Prosigna(R). *NPJ Breast Cancer*. 2021;7(1):15.
121. Bustamante Eduardo M, Popovici V, Imboden S, et al. Characterization of molecular scores and gene expression signatures in primary breast cancer, local recurrences and brain metastases. *BMC Cancer*. 2019;19(1):549.
122. Berchtold E, Vetter M, Gündert M, et al. Comparison of six breast cancer classifiers using qPCR. *Bioinformatics*. 2019;35(18):3412-3420.
123. Vallon-Christersson J, Häkkinen J, Hegardt C, et al. Cross comparison and prognostic assessment of breast cancer multigene signatures in a large population-based contemporary clinical series. *Sci Rep*. 2019;9(1):12184.
124. Chowdhury A, Pharoah PD, Rueda OM. Evaluation and comparison of different breast cancer prognosis scores based on gene expression data. *Breast Cancer Res*. 2023;25(1):17.
125. Picado O, Kwon D, Rojas K, et al. Impact of genomic assays on treatment and outcomes in locally advanced breast cancer. *Breast Cancer Res Treat*. 2022;194(2):433-447.
126. Varga Z, Sinn P, Seidman AD. Summary of head-to-head comparisons of patient risk classifications by the 21-gene recurrence Score (R) (RS) assay and other genomic assays for early breast cancer. *Int J Cancer*. 2019;45(4):882-893.
127. Stein RC, Dunn JA, Bartlett JM, et al. OPTIMA prelim: A randomised feasibility study of personalised care in the treatment of women with early breast cancer. *Health Technol Assess*. 2016;20(10):xxiii-xxix, 1-201.
128. Bartlett JMS, Bayani J, Marshall A, et al. Comparing breast cancer multiparameter tests in the OPTIMA prelim trial: no test is more equal than the others. *J Natl Cancer Inst*. 2016;108(9):djw050.
129. Sestak I, Dowsett M, Ferree S, Baehner FL, Cuzick J. Retrospective analysis of molecular scores for the prediction of distant recurrence according to baseline risk factors. *Breast Cancer Res Treat*. 2016;159(1):71-78.
130. Buus R, Yeo B, Brentnall AR, et al. Novel 18-gene signature for predicting relapse in ER-positive, HER2-negative breast cancer. *Breast Cancer Res*. 2018;20(1):103.
131. Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. *J Natl Cancer Inst*. 1996;88(20):1456-1466.
132. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *The Lancet*. 2005;365(9472):1687-1717.
133. Eliassen FM, Blåfjelldal V, Helland T, et al. Importance of endocrine treatment adherence and persistence in breast cancer survivorship: a systematic review. *BMC Cancer*. 2023;23(1):625.
134. Pagani O, Walley BA, Fleming GF, et al. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer: long-term follow-up of the combined TEXT and SOFT trials. *J Clin Oncol*. 2023;41(7):1376-1382.
135. Francis PA, Pagani O, Fleming GF, et al. Tailoring adjuvant endocrine therapy for premenopausal breast cancer. *N Engl J Med*. 2018;379(2):122-137.
136. Clinicaltrials.gov. Description of the OFSET trial (Evaluating the Addition of Adjuvant Chemotherapy to Ovarian Function Suppression Plus Endocrine Therapy in Premenopausal Patients With pN0-1, ER-Positive/HER2-Negative Breast Cancer and an Oncotype Recurrence Score Less Than or Equal to 25). Accessed November 13, 2023. Available at: [https://www.clinicaltrials.gov/study/NCT05879926?term=OFSET%20Chemo%20\(NRG-BR009\)%20randomized%20clinical%20trial&rank=1](https://www.clinicaltrials.gov/study/NCT05879926?term=OFSET%20Chemo%20(NRG-BR009)%20randomized%20clinical%20trial&rank=1)
137. Jeong JH, Jung SH, Wieand S. A parametric model for long-term follow-up data from phase III breast cancer clinical trials. *Stat Med*. 2003;22(3):339-352.
138. Jinih M, Relihan N, Corrigan MA, O'Reilly S, Redmond HP. Extended adjuvant endocrine therapy in breast cancer: evidence and update - a review. *Breast J*. 2017;23(6):694-705.
139. Mamounas EP, Bandos H, Rastogi P, et al. Ten-year update: NRG Oncology/National surgical adjuvant breast and bowel project B-42 randomized trial: extended letrozole therapy in early-stage breast cancer. *J Natl Cancer Inst*. 2023;115(11):1302-1309.
140. Foldi J, Tsiagianni A, Salganik M, et al. Persistence to extended adjuvant endocrine therapy following Breast Cancer Index (BCI) testing in women with early-stage hormone receptor-positive (HR +) breast cancer. *BMC Cancer*. 2023;23(1):606.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hyams DM, Bareket-Samish A, Rocha JEB, et al. Selecting postoperative adjuvant systemic therapy for early-stage breast cancer: An updated assessment and systematic review of leading commercially available gene expression assays. *J Surg Oncol*. 2024;1-22.
[doi:10.1002/jso.27692](https://doi.org/10.1002/jso.27692)