Clinical Validation of EndoPredict in Pre-Menopausal Women with ER-Positive, HER2-Negative Primary Breast Cancer

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ABSTRACT

Purpose: The EndoPredict prognostic assay is validated to predict distant recurrence and response to chemotherapy primarily in post-menopausal women with estrogen receptor–positive (ER+), HER2− breast cancer. This study evaluated the performance of EndoPredict in pre-menopausal women.

Experimental Design: Tumor samples from 385 pre-menopausal women with ER+, HER2− primary breast cancer (pT1-3, pN0-1) who did not receive chemotherapy in addition to endocrine therapy were tested with EndoPredict to produce a 12-gene EP molecular score and an integrated EPclin score that includes pathologic tumor size and nodal status. Associations of molecular and EPclin scores with 10-year distant recurrence–free survival (DRFS) were evaluated by Cox proportional hazards models and Kaplan–Meier analysis.

Results: After a median follow-up of 9.7 years, both the EP molecular score and the molecular-clinicopathologic EPclin score were associated with increased risk of distant recurrence [HR, 1.33; 95% confidence interval (CI), 1.18–1.50; $P = 7.2 \times 10^{-6}$; HR, 3.58; 95% CI, 2.26–5.66; $P = 9.8 \times 10^{-8}$, respectively]. Both scores remained significant after adjusting for clinical factors in multivariate analysis. Patients with low-risk EPclin scores (64.7%) had significantly improved DRFS compared with high-risk patients (HR, 4.61; 95% CI, 1.40–15.17; $P = 4.2 \times 10^{-3}$). At 10 years, patients with low-risk and high-risk EPclin scores had a DRFS of 97% (95% CI, 93%–99%) and 76% (95% CI, 67%–82%), respectively.

Conclusions: The EPclin score is strongly associated with DRFS in pre-menopausal women who received adjuvant endocrine therapy alone. On the basis of these data, pre-menopausal women with EPclin low-risk breast cancer may be treated with endocrine therapy only and safely forgo adjuvant chemotherapy.

Introduction

Luminal estrogen receptor–positive (ER+), HER2− breast cancer is the most commonly diagnosed form of breast cancer. Fortunately, women with luminal breast cancer have some of the most favorable outcomes particularly if disease is detected and treatment is initiated at an early stage. Currently, all women with hormone receptor–positive breast cancer are recommended to receive at least 5 years of adjuvant endocrine therapy (1, 2). A proportion of women with luminal breast cancer that are likely to have higher risk may also receive adjuvant chemotherapy. Treatment regimens of patients with luminal breast cancer are determined on the basis of a number of factors, including a patient's individual risk of disease recurrence and/or predicted benefit from chemotherapy (1). Traditionally, the risk has been determined using a number of clinical factors such as tumor size, nodal status, hormone receptor status, age, and others. In the last two decades, molecular signatures have been developed to provide more objective information about the risk of recurrence without chemotherapy (i.e., prognostic markers). More recently, they have been shown to also predict a patient's benefit from chemotherapy (i.e., predictive markers).

There are currently a number of commercially available prognostic and predictive assays to inform risk of recurrence and benefit of chemotherapy, respectively, for patients with localized luminal breast cancer (3). One such test is the Oncotype DX Breast Recurrence Score test, the only test for which prospective randomized phase III studies have found that genomic low-risk patients do not benefit from adjuvant chemotherapy. These studies have shown that post-menopausal patients with up to three positive lymph nodes and a recurrence score (RS)≤25, and patients who are pre-menopausal with node-negative disease and a RS<16 do not have a better outcome with chemotherapy compared with endocrine therapy alone (4, 5). Two other retrospective studies with samples from prospective randomized trials demonstrated that patients with a high-risk RS (RS≥31) benefit from adjuvant chemotherapy (6, 7). Another test is the 12-gene EndoPredict assay that produces an EP molecular score (also referred to as 12-gene molecular score) that is combined with tumor size and nodal status to produce a clinicomolecular score (namely EPclin score; ref. 8). Previous studies have demonstrated that EPclin score is prognostic of recurrence risk in breast cancer (8–11). A retrospective indirect comparison of EPclin score in patients treated with chemotherapy

Translational Relevance

Treatment decisions for pre-menopausal women with estrogen receptor–positive (ER⁺), HER2⁻ localized breast cancer are based on factors such as tumor grade, tumor size, age, and Ki-67 status as well as multigene signatures. The majority of development and validation studies supporting multigene signatures have been conducted in cohorts of primarily post-menopausal women. Recent literature, however, has suggested that the performance of the multigene signatures may differ depending on menopausal status. It is therefore necessary to demonstrate clinical validity of multigene signatures for pre-menopausal women with ER⁺, HER2⁻ breast cancer.

plus endocrine therapy versus endocrine therapy alone showed that the EPclin score, but not the EP molecular score, predicted chemotherapy benefit (12). The majority of the validation and utility work performed for the EndoPredict assay has used cohorts of primarily post-menopausal women, a common practice for this type of test. Recent literature, however, has suggested that the performance of these breast prognostic assays may differ depending on menopausal status. Three recent prospective randomized studies, the Oncotype DX TAILORx, and RxPonder trials and the Mammaprint MINDACT trial (4, 5, 13, 14), have indicated differences in the prognostic ability and predictive power of molecular signatures in women based on their menopausal status. This study aimed to evaluate the association between the EPclin score and 10-year distant recurrence within a population of pre-menopausal women with ER⁺, HER2⁻ early-stage breast cancer who received endocrine therapy but did not receive chemotherapy.

Materials and Methods

Study cohort

Archived formalin-fixed paraffin-embedded (FFPE) sections of early-stage breast cancer samples were obtained from the Bank of Cyprus Oncology Centre (BOCOC) and the Nottingham University Hospitals NHS Trust (NHU). Samples from BOCOC were collected between 2003 and 2016, and samples from NHU were collected between 1990 and 1998. All samples were collected from unselected cohorts of patients with breast cancer who were intended to be treated with adjuvant endocrine therapy alone with no neoadjuvant or adjuvant chemotherapy given (see **Table 1** for treatment details). Samples were eligible for inclusion in the study if the patient was at least 18 years of age and pre-menopausal at the time of breast cancer diagnosis and had ER⁺, HER2⁻ localized disease with a pathologic tumor size ≤pT3 and node status of pN0-1. Menopausal status was based on the final menstrual period. Pre-menopausal patients were defined as women with regular menstruation (or amenorrhea less than 8 weeks) at the time of breast cancer diagnosis. Patients who received chemotherapy were excluded. Patients were also excluded if clinical data necessary to calculate the EPclin score (tumor size or nodal status) were unavailable. Samples with insufficient tumor volume or quality to produce a passing EP molecular score were excluded from the final study cohort. Central pathology review was performed independently in the BOCOC and NHU tissue biobanks. The research was conducted according to the Declaration of Helsinki, and written informed consent was obtained from all patients. This work obtained ethics approval to use the human tissue samples either by the National

Table 1. Patient characteristics.

Characteristic	BOCOC (n = 276)	NHU (n = 109)	Total (n = 385)
Median follow-up time (y)	8.4	17.7	9.7
Age at diagnosis (y), mean (SD)	46.8 (4.5)	45.7 (4.9)	46.5 (4.7)
Tumor size (cm), mean (SD)	1.3 (0.7)	2.2 (0.9)	1.6 (0.8)
Tumor size (TNM stage), N (%)			
pT1a	21 (7.6%)	0 (0%)	21 (5.5%)
pT1b	91 (33.0%)	6 (5.5%)	97 (25.2%)
pT1c	142 (51.4%)	49 (45.0%)	191 (49.6%)
pT2	22 (8.0%)	53 (48.6%)	75 (19.5%)
pT3	0 (0%)	1 (0.9%)	1 (0.3%)
Tumor grade, N (%)			
I	76 (27.5%)	9 (8.3%)	85 (22.1%)
II	187 (67.8%)	52 (47.7%)	239 (62.1%)
III	10 (3.6%)	48 (44.0%)	58 (15.1%)
Missing	3 (1.1%)	0 (0%)	3 (0.8%)
Nodal status, N (%)			
Positive (pN1)	16 (5.8%)	46 (42.2%)	62 (16.1%)
Negative (pN0)	260 (94.2%)	63 (57.8%)	323 (83.9%)
Ki-67 expression (continuous), mean (SD)	11.0 (10.6)	24.2 (22.4)	14.7 (16.0)
Ki-67 expression (categorical), N (%)			
≤5%	118 (42.8%)	14 (12.8%)	132 (34.3%)
6%–29%	129 (46.7%)	61 (56.0%)	190 (49.4%)
≥30%	19 (6.9%)	28 (25.7%)	47 (12.2%)
Missing	10 (3.6%)	6 (5.5%)	16 (4.2%)
ER expression (%), mean (SD)	81.9 (17.0)	87.2 (17.3)	83.4 (17.2)
PgR expression (%), mean (SD)	80.9 (25.4)	72.0 (39.9)	78.5 (30.3)
Adjuvant endocrine therapy, N (%)			
Tamoxifen	97 (35.1%)	67 (61.5%)	164 (42.6%)
Tamoxifen + OFS	175 (63.4%)	35 (32.1%)	210 (54.5%)
Other[a]	2 (0.7%)	5 (4.6%)	7 (1.8%)
None/NA[b]	2 (0.7%)	2 (1.8%)	4 (1.0%)
OFS (goserelin), N (%)	177 (64.1%)	37 (33.9%)	214 (55.6%)
(Neo)adjuvant chemotherapy, N (%)			
Yes	0 (0%)	0 (0%)	0 (0%)
No	276 (100%)	109 (100%)	385 (100%)

Abbreviations: BOCOC, Bank of Cyprus Oncology Centre; ER, estrogen receptor; NA, not available; NHU, Nottingham University Hospitals NHS Trust; OFS, ovarian function suppression; PgR, progesterone receptor; TNM, tumor, nodes, metastases.
[a]Includes treatment with aromatase inhibitor, aromatase inhibitor + OFS, and OFS alone.
[b]Includes patients who were intended to be treated with endocrine therapy but either did not receive it due to anticipated side effects or endocrine therapy details were not available.

Bioethics Committee in Cyprus or by the North West Greater Manchester Central Research Ethics Committee.

EndoPredict testing

All samples were retrospectively tested in one central laboratory blinded to clinical and outcome data with the 12-gene EndoPredict test, as previously described (8, 15). Briefly, RNA was extracted from 10-μm sections of FFPE with at least 30% invasive tumor. The expression of 8 genes-of-interest (*AZGP1*, *BIRC5*, *DHCR7*, *IL6ST*, *MGP*, *RBBP8*, *STC2*, and *UBE2C*), 3 normalization genes (*CALM2*, *OAZ1*, and *RPL37A*), and 1 DNA control gene (*HBB*) was measured in triplicate by quantitative RT-PCR. The EP molecular score was then calculated using the normalized expression of the genes-of-interest. Patients with 12-gene EP molecular scores under 5 were

considered low risk for distant recurrence and scores at or above 5 were considered high risk (8). The EP molecular score was combined with patient-specific tumor size and nodal status to calculate the combined clinicomolecular EPclin score, as described previously (8). Patients with EPclin scores less than 3.32867 were considered to be at low risk for distant recurrence, and scores greater than or equal to 3.32867 were considered to be at high risk of distant recurrence (8).

Statistical analysis

The primary analysis evaluated the association of the EPclin score as a continuous variable with 10-year distant recurrence in pre-menopausal women. Multiple secondary analyses tested the following objectives: univariate associations of the categorical EPclin score, the EP score (continuous and categorical), and individual clinical variables with 10-year distant recurrence; the association of EPclin score with 10-year distant recurrence after multivariate adjustment for clinical variables; and the association of the EP score with 10-year distant recurrence after multivariate adjustment for clinical variables and Kaplan–Meier estimates of distant recurrence–free survival (DRFS) in the EPclin and EP risk categories.

The primary outcome was 10-year DRFS, which was defined as the time from the initial breast cancer procedure (i.e., mastectomy or wide local excision to remove cancer) to the first distant recurrence. Distant recurrence was defined as metastatic disease, excluding contralateral disease, and locoregional and ipsilateral recurrences. Death before distant recurrence was treated as a censoring event. As an exploratory endpoint, 10-year breast cancer–free interval (BCFI) was used. BCFI was defined as the time from the date of the initial breast cancer procedure until the date of either the first distant recurrence, local recurrence, regional recurrence, contralateral breast cancer, or breast cancer–related death, whichever came first (16). Besides EPclin and EP molecular score, clinical variables of interest included age at diagnosis, tumor size (≤1 cm, >1–≤2 cm, >2–≤5 cm, >5 cm), tumor grade (I, II, III, missing), nodal status (positive, negative), Ki-67 expression (≤5%, 6%–29%, ≥30%, missing), and continuous ER expression (%) and progesterone receptor (PgR) expression (%). Positive nodal status was defined as one or more nodes with no individual having more than 3 positive nodes. The categories of Ki-67 expression (≤5%, 6%–29%, and ≥30%) were based on recommendations from the International Ki67 in Breast Cancer Working Group (17).

Univariate Cox proportional hazards models were used to evaluate the association between 10-year DRFS and each clinical variable. A multivariate Cox proportional hazards model, including nodal status and clinical variables with statistically significant univariate associations, was used to evaluate the independent prognostic power of the EP molecular score. As the EPclin score includes nodal status and tumor size, multivariate models included all clinical variables with statistically significant univariate associations except for nodal status and tumor size. Because of differences in cohort characteristics between the BOCOC and NHU cohorts (**Table 1**), all Cox proportional hazards analyses were stratified by site.

DRFS was evaluated using the Kaplan–Meier method to estimate probabilities of 10-year DRFS with 95% confidence intervals (CI). Subset analyses were performed by cohort, nodal status, and ovarian function suppression (OFS) as determined by the use of goserelin. P values were calculated from likelihood ratio χ^2 statistics and reported as two-sided. Statistical significance was defined as $P < 0.05$. The database, which included pathology, clinical, outcome, and EndoPredict testing data, was centrally reviewed before statistical analysis. All analyses were performed using R version 3.5.3 or higher

(R Foundation for Statistical Computing) and SAS software version 9.2 or higher (SAS Institute Inc.).

Data availability

The data generated in this study are available upon reasonable request from the corresponding author.

Results

Patient population

In total, 417 patients across two sites were identified for inclusion in this study and had samples analyzed with EndoPredict. One patient was excluded because of a non-invasive carcinoma in the tissue, and 5 patients were excluded because they were subsequently found to have post-menopausal status. Of the 411 remaining patients, 408 had tumor size and nodal status available to calculate EPclin scores. Of these, 385 had a valid EndoPredict test result and were included in the final study population: 276 from BOCOC and 109 from NHU (**Table 1**). The NHU sub-cohort tended to have a more aggressive cancer phenotype with a higher proportion of the population being grade III (44.0%), having nodal involvement (42.2%), and having a higher average Ki-67 expression (mean 24.2) compared with the BOCOC cohort (3.6%, 5.8%, and mean 11.0, respectively). The reason for the lower baseline risk of the BOCOC cohort was that patients with grade III disease, larger tumors, or node-positive disease were automatically considered high risk and, therefore, received chemotherapy and were excluded from this study.

The median follow-up time for the full cohort was 9.7 years. Overall, 64.7% of the full cohort was classified as low risk and 35.3% as high-risk by EPclin score. Of patients with node-negative disease ($N = 323$), 73.4% were low risk by EPclin score, whereas 26.6% were high risk. Conversely, of patients with node-positive disease ($N = 62$), 19.4% were low risk by EPclin score and 80.6% were high risk. During the follow-up period, 47 patients (12.2%) experienced an event, 35 of which were a distant recurrence. The other twelve events were also breast cancer related (i.e., local recurrence, loco-regional recurrence, contralateral breast cancer). There were 4 and 31 distant recurrences in patients with low- and high-risk EPclin scores, respectively, and 0 and 35 in patients with low- and high-risk EP molecular scores.

Associations with distant recurrence

Results of the primary analysis using univariate Cox proportional hazards models showed that the continuous EPclin score was strongly associated with the risk of distant recurrence within 10 years of diagnosis (HR, 3.58; 95% CI, 2.26–5.66; $P = 9.8 \times 10^{-8}$; Supplementary Table S1). The EPclin score was significantly prognostic for both women with node-negative (HR, 2.61; 95% CI, 1.53–4.46; $P = 4.8 \times 10^{-4}$) and node-positive disease (HR, 6.29; 95% CI, 2.63–15.06; $P = 3.9 \times 10^{-5}$). The EPclin score as a categorical variable (HR, 4.61; 95% CI, 1.40–15.17; $P = 4.2 \times 10^{-3}$) and EP molecular score as both a continuous (HR, 1.33; 95% CI, 1.18–1.50; $P = 7.2 \times 10^{-6}$) and categorical (HR incalculable due to zero events in the low-risk category, $P = 9.8 \times 10^{-5}$) variable were significantly associated with 10-year distant recurrence. A similar result was found when excluding 4 patients who were intended to be treated with endocrine therapy but either did not receive it due to anticipated side effects or endocrine therapy details were not available (Supplementary Table S2; Supplementary Fig. S1). Besides EPclin score and EP molecular score, in univariate analysis we also observed significant associations between 10-year distant recurrence and age, tumor size, tumor grade, Ki-67, and PgR expression (Supplementary Table S1).

Table 2. Multivariate Cox proportional hazards analysis: EPclin score.

Variable	Reference	Level	HR (95% CI)	P
EPclin score (continuous)	—	—	2.91 (1.70–4.97)	8.3×10^{-5}
Age at diagnosis (continuous)	—	—	0.96 (0.90–1.02)	0.18
Tumor grade (categorical)	I	II	8.98×10^7 (0–∞)	5.8×10^{-2}
		III	1.01×10^8 (0–∞)	
		Missing	0.72 (0–∞)	
Ki-67 (categorical)	≤5%	6%–29%	2.51 (0.57–10.97)	0.41
		≥30%	1.61 (0.33–7.97)	
		Missing	3.88 (0.33–46.14)	
ER expression (%; continuous)	—	—	0.99 (0.97–1.02)	0.59
PgR expression (%; continuous)	—	—	1.00 (0.99–1.01)	0.69

Note: Model is stratified by site; analysis includes 34/381 (Events/N). PgR expression was missing for 4 patients, one of whom had a distant recurrence within 10 years.
Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

To determine whether the continuous EPclin and the continuous EP molecular scores were prognostic independent of other clinical factors, multivariate Cox proportional hazards models were used. In multivariate analyses, the EPclin score was strongly associated with the risk of distant recurrence within 10 years of diagnosis (HR, 2.91; 95% CI, 1.70–4.97; $P = 8.3 \times 10^{-5}$) independent of age at diagnosis, tumor grade, Ki-67, and level of hormone receptor expression (**Table 2**). Similarly, EP molecular score, tumor size, nodal status, and tumor grade were all independently associated with distant recurrence ($P < 0.05$; **Table 3**).

DRFS and BCFI

The Kaplan–Meier analysis was used to estimate DRFS (**Figs. 1–3**). For the full study cohort, patients with low-risk EPclin scores had a 10-year DRFS of 97% (95% CI, 93%–99%) whereas patients with high-risk EPclin scores had a 10-year DRFS of 76% (95% CI, 67%–82%; $P = 4.2 \times 10^{-3}$; **Fig. 1A**). DRFS was similar when risk classification was based on EP molecular score alone (**Fig. 2**). Overall, patients with high-risk EP molecular scores (10-year DRFS, 84%; 95% CI, 78%–88%) had worse outcomes than patients with low-risk EP molecular scores (10-year DRFS, 100%; $P = 9.8 \times 10^{-5}$; **Fig. 2A**). Conversely, there were no events among patients with low-risk EP molecular scores, which suggest a higher probability of survival.

Similar 10-year DRFS was observed in patients with low- and high-risk EPclin and EP molecular scores when excluding 4 patients who were intended to be treated with endocrine therapy but either did not receive endocrine therapy or endocrine therapy details were not available (Supplementary Table S2; Supplementary Fig. S1).

When DRFS was evaluated in sub-populations of women with node-negative ($N = 323$) and node-positive ($N = 62$) disease, similar results were observed. Similar to the whole cohort, women with node-negative disease and low-risk EPclin scores had good outcomes (10-year DRFS, 97%; 95% CI, 93%–99%). This was significantly higher than women with high-risk EPclin scores (10-year DRFS, 76%; 95% CI, 64%–84%; $P = 1.5 \times 10^{-2}$; **Fig. 1B**). Similarly, among women with node-negative disease, those with high-risk EP molecular scores had a 10-year DRFS of 86% (95% CI, 79%–91%), which was significantly lower than DRFS in women with low-risk EP molecular scores (no events observed; 10-year DRFS, 100%; $P = 2.1 \times 10^{-3}$; **Fig. 2B**).

There were no events among women with node-positive disease and low-risk EPclin or EP molecular scores, so 10-year DRFS was 100% (**Figs. 1C and 2C**). Ten-year DRFS for women with node-positive disease and high-risk EPclin scores was similar to those with high-risk EP molecular scores (75%; 95% CI, 61%–85% and 75%; 95% CI, 60%–85%, respectively). However, increased risk of distant recurrence in patients with node-positive disease and high-risk EPclin scores did not

Table 3. Multivariate Cox proportional hazards analysis: EP molecular score.

Variable	Reference	Level	HR (95% CI)	P
EP molecular score (continuous)	—	—	1.24 (1.05–1.47)	1.2×10^{-2}
Tumor size (categorical)	≤1 cm	>1 to ≤2 cm	2.58×10^8 (0–∞)	8.5×10^{-4}
		>2 to ≤5 cm	7.86×10^8 (0–∞)	
		>5 cm	4.96 (0–∞)	
Nodal status (categorical)	Negative	1–3 nodes	2.85 (1.14–7.12)	2.8×10^{-2}
Age at diagnosis (continuous)	—	—	0.96 (0.90–1.03)	0.23
Tumor grade (categorical)	I	II	3.97×10^8 (0–∞)	1.9×10^{-2}
		III	4.95×10^8 (0–∞)	
		Missing	6.65×10^7 (0–∞)	
Ki-67 (categorical)	≤5%	6%–29%	2.48 (0.56–10.89)	0.36
		≥30%	2.12 (0.41–10.92)	
		Missing	10.18 (0.75–138.94)	
ER expression (%; continuous)	—	—	0.99 (0.97–1.01)	0.53
PgR expression (%; continuous)	—	—	0.99 (0.98–1.00)	0.23

Note: Model is stratified by site; analysis includes 34/381 (Events/N). PgR expression was missing for 4 patients, one of whom had a distant recurrence within 10 years.
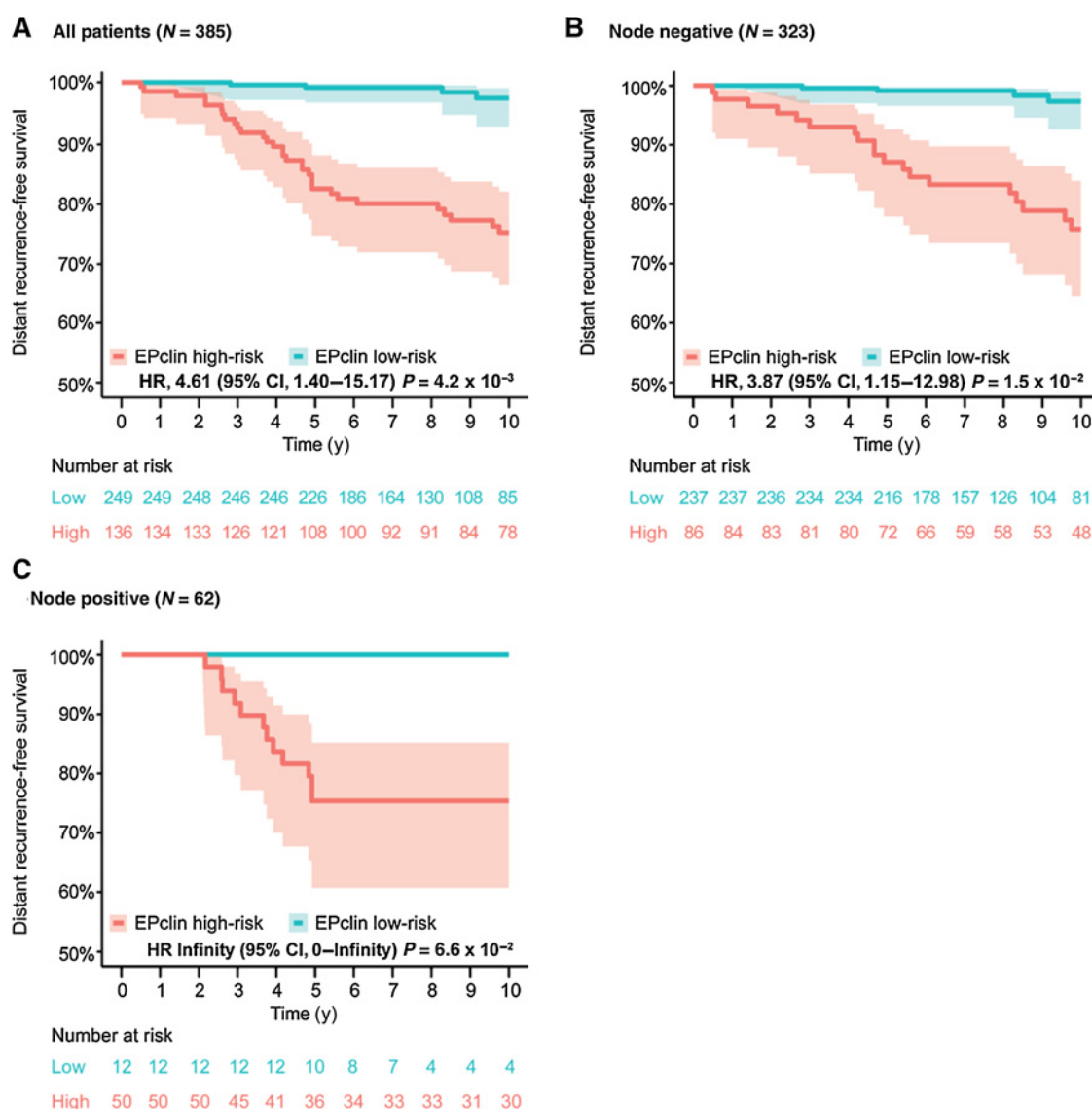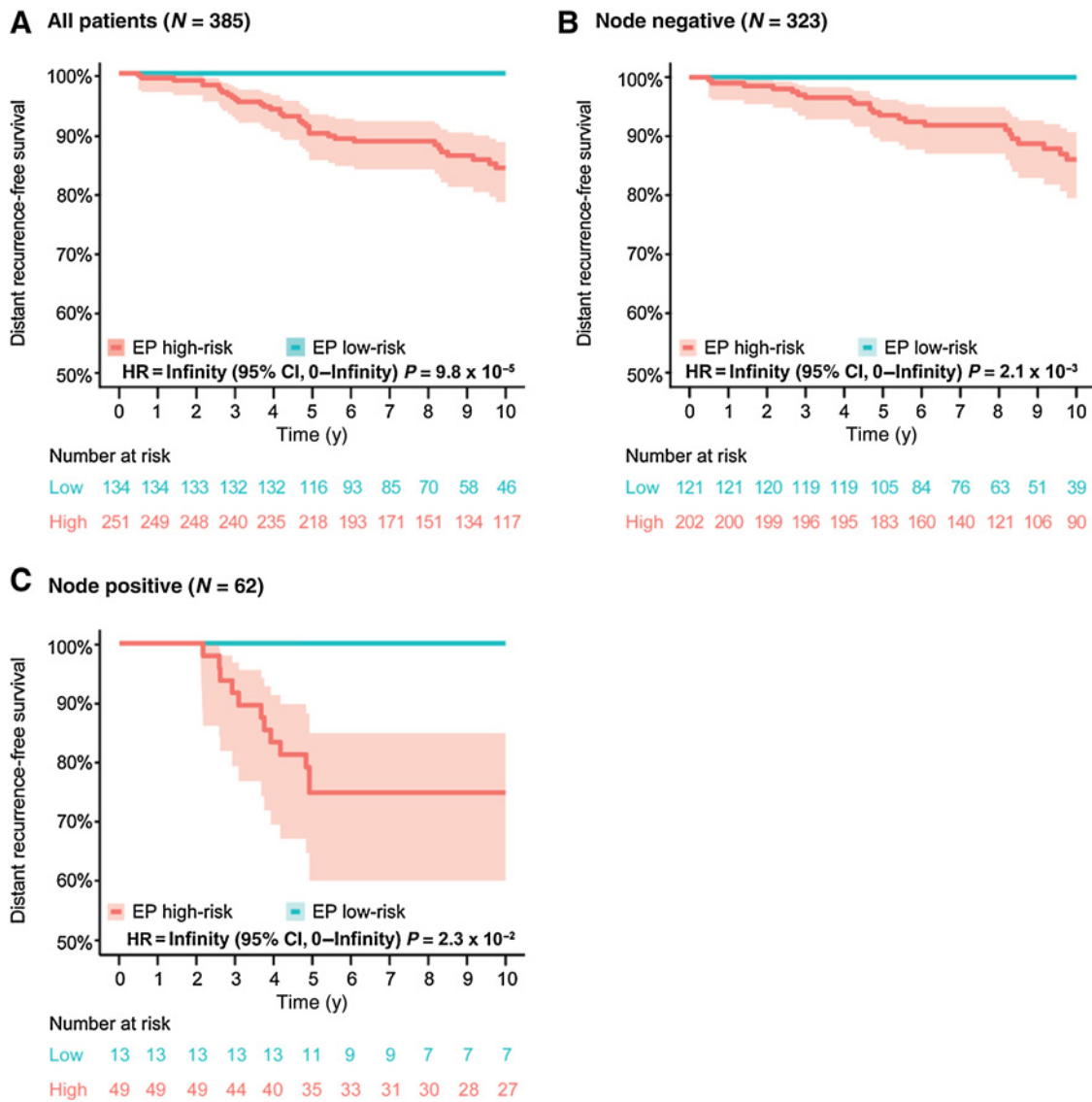Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

Figure 1.
Distant recurrence–free survival over 10 years by EPclin Score for (**A**) the full cohort, (**B**) node-negative patients, and (**C**) node-positive patients.

reach statistical significance compared with the patients with low-risk EPclin scores ($P = 6.6 \times 10^{-2}$), possibly due to the small sample size. Patients with high-risk EP molecular scores and node-positive disease had a significantly increased risk of distant recurrence compared with patients with low-risk EP molecular scores ($P = 2.3 \times 10^{-2}$).

Exploratory subgroup analyses were performed in patients who had not received OFS ($N = 171$) and those who had ($N = 214$). For patients with low-risk EPclin scores who did not receive OFS, the 10-year DRFS was 99% (95% CI, 93%–100%), which was significantly higher than that of the patients with high-risk EPclin scores who had a 10-year DRFS of 80% (95% CI, 67%–88%; $P = 3.4 \times 10^{-2}$; **Fig. 3A**). A similar but non-significant result was found among women who were treated with OFS. Among women treated with OFS, those who had a low-risk EPclin score had a 10-year DRFS of 96% (95% CI, 88%–99%), whereas those with a high-risk EPclin score had 10-year DRFS of 72% (95% CI, 59%–82%; $P = 0.11$; **Fig. 3B**).

In a further exploratory analysis evaluating BCFI, including all recurrence events in all patients, patients with low-risk EPclin and EP molecular scores were significantly different from patients with high-risk scores (EPclin: HR, 3.63; 95% CI, 1.57–8.38; $P = 1.5 \times 10^{-3}$; EP molecular: HR, 3.43; 95% CI, 1.21–9.75; $P = 6.9 \times 10^{-3}$; Supplementary Figs. S2A and S3A). Patients with low-risk EPclin and EP molecular scores had a 10-year breast cancer–free rate of 94% (95% CI, 89%–97%) and 95% (95% CI, 88%–98%), respectively, whereas patients with high-risk EPclin and EP molecular scores had a 10-year breast cancer–free rate of 71% (95% CI, 62%–78%) and 80% (95% CI, 74%–85%).

Discussion

This study evaluated the performance of the validated 12-gene EndoPredict breast prognostic assay in a population of pre-

Figure 2.
Distant recurrence–free survival over 10 years by EP molecular score for (**A**) the full cohort, (**B**) node-negative patients, and (**C**) node-positive patients.

menopausal women. It showed that both the EP molecular and the combined clinicomolecular EPclin scores were prognostic of 10-year distant recurrence in pre-menopausal women. EPclin identified 65% of pre-menopausal patients with a 10-year distant recurrence risk of <4% treated with endocrine therapy alone who safely avoided adjuvant chemotherapy.

Our data are consistent with previous work in populations of primarily post-menopausal women, indicating that menopausal status does not impact the performance and the utility of this test (Supplementary Table S3). In three independent prospective–retrospective clinical validation trials, including patients with breast cancer from the three phase III studies ABCSG-6, ABCSG-8, and TransATAC, EPclin identified approximately two thirds of low-risk patients with a 10-year distant recurrence risk between 4% and 5.8% without adjuvant chemotherapy (8–10). Another study, including pre- and post-menopausal patients who had been treated with endocrine

therapy and chemotherapy, indicated that there is no difference in the prognostic power of the test between pre- and post-menopausal disease (11).

Multivariate analyses demonstrated that the EP molecular score was prognostic, independent of nodal status. Moreover, node-negative and node-positive patients with breast cancer who were classified as low-risk by EPclin had a 10-year DRFS of 97% and 100%, respectively, though the sample size of the node-positive subgroup was relatively small ($N = 62$). This is also consistent with results in patients with post-menopausal disease. As presented in previous clinical validation studies, regardless of nodal status, patients with low-risk EPclin scores have a risk of distant recurrence between 4% and 6% (9, 10). This suggests that pre- and post-menopausal patients with breast cancer who have low-risk EPclin scores can safely forgo adjuvant chemotherapy independent of nodal status.
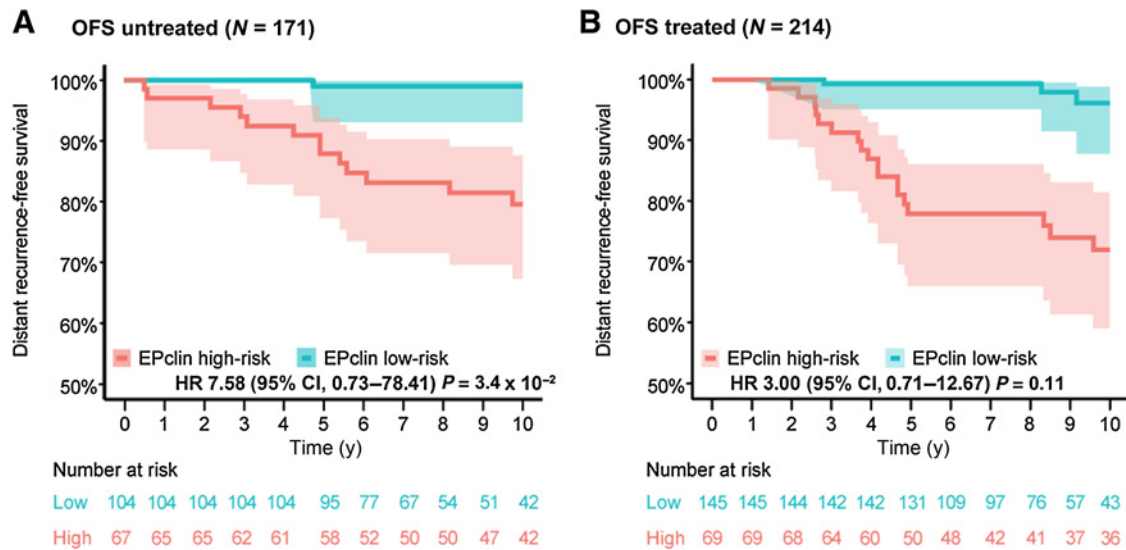
Figure 3.
Distant recurrence–free survival over 10 years by EPclin Score for (**A**) patients who were not treated with ovarian function suppression (OFS) and (**B**) patients who were treated with OFS.

Treatment of pre-menopausal women with localized breast cancer can be challenging, due to their longer life expectancy and differences in disease biology and outcomes (18–22). Therefore, it is critical to ensure that disease does not recur, while also mitigating long-lasting side effects in these patients. Many patients with early-stage luminal (ER$^+$, HER2$^-$) breast cancer are able to forgo chemotherapy, a treatment associated with many transient and chronic side-effects, and prognostic/predictive assays are important tools to inform the appropriate treatment decisions. However, compared with post-menopausal women, the prognosis of pre-menopausal women tends to be poorer, resulting in more use of adjuvant chemotherapy in this group (18–20). Accurate prognosis of risk combined with a selection of the appropriate endocrine regimen, which may differ from the endocrine regimen for post-menopausal women, is therefore critical in achieving long-lasting recurrence-free survival in pre-menopausal patients. OFS has been shown to improve outcomes and reduce the risk of recurrence in pre-menopausal women (23–26). The addition of OFS to tamoxifen results in significantly higher rates of both disease-free survival and overall survival than tamoxifen alone (27, 28), such that patients with breast cancer who have a higher risk of recurrence are routinely treated with OFS. In our study, 55.6% of patients were treated with OFS. Exploratory subgroup analyses by OFS treatment status demonstrated that patients with low-risk EPclin scores in both subgroups had a good 10-year DRFS of 96% (with OFS) and 99% (without OFS), respectively.

In the prospective TAILORx trial evaluating patients with node-negative disease, the investigators observed that the treatment benefit of adjuvant chemotherapy varied on the basis of combinations of their 21-gene RS (Oncotype DX) with menopausal status, indicating that menopausal status affected the performance of the test (4, 14). Similarly, the randomized RxPonder trial evaluating patients with node-positive disease found that a differential treatment effect of chemotherapy benefitted pre- and post-menopausal patients based on RS (5). However, seeing as only 16% of patients with endocrine therapy had received OFS, it is not clear whether the observed chemotherapy benefit in RxPonder was a direct cytotoxic effect or

an indirect effect due to chemotherapy-induced OFS. In another prospective trial evaluating the 70-gene Mammaprint test (MIND-ACT), women ≤50 years of age, with up to 3 positive lymph nodes, and a high clinical but low genomic risk had a benefit from chemotherapy (13). The findings in these prospective randomized trials are reflected in the recent update to the American Society of Clinical Oncology guidelines, which now recommend Oncotype DX, but not Mammaprint, for pre-menopausal node-negative disease (29). For pre-menopausal node-positive disease, no gene expression tests are currently recommended. Other available gene expression tests such as Prosigna [Risk of Recurrence (ROR) score] or Breast Cancer Index only have limited data in exclusively pre-menopausal cohorts; thus, the clinical utility in this subgroup is unclear based on current evidence (29). One study assessed Prosigna in pre-menopausal women and showed that ROR score was associated with prognosis in this patient group (30). However, it is difficult to translate these findings to clinical applicability, as the cohort included patients with triple-negative or HER2-positive disease, and patients did not receive endocrine therapy. The current study is the first to evaluate Endo-Predict in an exclusively pre-menopausal cohort of patients who received endocrine therapy and did not receive chemotherapy. With a very low risk of distant recurrence, pre-menopausal patients with low-risk EPclin scores were able to safely forego chemotherapy. EndoPredict may have further prognostic implications in this patient group. Moreover, based on the exploratory results in our study, there seems to be no detectable difference between 10-year DRFS in patients with low-risk EPclin scores who received OFS treatment and those who did not. Therefore, one might speculate that patients with low-risk EPclin scores do not benefit from OFS and can proceed with 5 years of tamoxifen treatment alone, although the effectiveness of this plan would need to be evaluated in a larger cohort.

The strength of this study is that it is a defined cohort of patients with ER$^+$, HER2$^-$ breast cancer who were not treated with chemotherapy. Moreover, the long median follow-up time of almost 10 years is important in light of the relevance of late recurrence in ER$^+$ breast cancer. However, this study has some limitations. First, the

study cohort was derived from two separate sites with different patient populations, which is reflected in the differences in the cohort characteristics. The analyses were stratified by site as appropriate to account for these differences. Second, there is a relatively small number of distant recurrences in this cohort (35 events), including only four events in the patients with low-risk EPclin scores, which likely caused some analyses to lack statistical significance. Third, this study was not randomized and focused solely on the prognostic ability of the scores as this population received endocrine therapy alone. Although the predictive capacity of this test was not evaluated here, a previous study that established the predictive ability used a mixed cohort containing both pre- and post-menopausal women (12). In addition, a substantial absolute benefit from chemotherapy in the patients with low-risk EPclin or EP molecular scores who had a 10-year distant recurrence risk of 0% and 3%, respectively, is highly unlikely.

Overall, these results support the use of this test for all women with early-stage ER$^+$, HER2$^-$ localized breast cancer, regardless of menopausal status. As approximately 29% of new breast cancer cases occur in patients ages 54 or younger (31), use of this test among pre-menopausal women will provide important information to inform personalized treatment selection for a large proportion of women with this disease. Appropriate selection of adjuvant endocrine therapy with or without chemotherapy is critical for the long-term survival of patients with breast cancer and the highest quality of life possible.

Authors' Disclosures

Authors' Contributions

Acknowledgments

Note

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