Published OnlineFirst May 7, 2019; DOI: 10.1158/1078-0432.CCR-19-0376

Precision Medicine and Imaging

Clinical Cancer Research

Prediction of Distant Recurrence Using EndoPredict Among Women with ER$^+$, HER2$^-$ Node-Positive and Node-Negative Breast Cancer Treated with Endocrine Therapy Only

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Abstract

Purpose: Prognostic molecular assays may aid in treatment decisions for women with estrogen receptor (ER)-positive, HER2-negative breast cancer. The prognostic value of a 12-gene expression assay (EndoPredict) was reevaluated in the combined ABCSG-6/8 cohorts with longer clinical follow-up.

Experimental Design: EndoPredict (EP; molecular score, EPclin score) was evaluated in women with ER-positive, HER2-negative node-positive and node-negative breast cancer who received 5 years of endocrine therapy only (median follow-up, 9.6 years; $N = 1{,}702$). Distant recurrence-free rate (DRFR; 95% confidence interval) was assessed 10 and 15 years after diagnosis.

Results: Overall, 62.6% of patients had low-risk EPclin scores with significantly improved DRFR relative to high-risk patients (HR, 4.77; 95% CI, 3.37–6.67; $P < 0.0001$). Ten-year DRFR (0–10 years) was improved among patients with low-risk versus high-risk EPclin scores in the full cohort [95.5% (94.1%–97.0%) vs. 80.3% (76.9%–83.9%)] as well as for patients with node-negative disease [95.5% (94.0%–97.1%) vs. 87.0% (82.6%–91.7%)] or with 1 to 3 positive nodes [95.6% (92.2%–99.1%) vs. 80.9% (75.9%–86.1%)]. The molecular and EPclin scores were significant predictors of DRFR after adjusting for clinical variables, regardless of nodal status. Similar results were observed for late recurrence (5–15 years; HR, 4.52; 95% CI, 2.65–7.72; $P < 0.0001$). The EPclin score significantly added prognostic information to a late metastasis nomogram (CTS5 score; $P < 0.001$).

Conclusions: This study demonstrates that EPclin can identify patients at low risk for early or late recurrence who may safely forgo adjuvant chemotherapy or extended endocrine therapy, respectively, regardless of nodal status.

Introduction

Professional society guidelines recommend that women with estrogen receptor (ER)-positive, HER2-negative breast cancer receive adjuvant endocrine therapy (1, 2). Initial treatment may also include adjuvant chemotherapy (1, 2). Decisions regarding the addition of chemotherapy are based on the risk of distant recurrence, as many women with low-risk disease can safely avoid chemotherapy. This risk assessment has historically been based solely on clinical factors, including tumor stage and grade, nodal status, and patient age.

More recently, prognostic molecular assays validated to predict the 10-year risk of distant recurrence among women with ER-positive, HER2-negative breast cancer have been integrated into initial treatment decisions regarding adjuvant chemotherapy (3, 4). Despite the fact that these molecular assays have been shown to provide improved prognostic information over clinical factors alone, some women are still considered high-risk based only on clinical factors.

Women with ER-positive, HER2-negative breast cancer now face a second treatment decision 5 years after diagnosis: whether or not to continue endocrine therapy. Conflicting data have emerged regarding the efficacy of extending endocrine therapy beyond 5 years and, as a result, the optimal duration and regimen of adjuvant endocrine therapy is not clear. Several trials have

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2019;25:1–8

doi: 10.1158/1078-0432.CCR-19-0376

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Published OnlineFirst May 7, 2019; DOI: 10.1158/1078-0432.CCR-19-0376

Translational Relevance

Women with estrogen receptor (ER)-positive, HER2-negative breast cancer are faced with treatment decisions at the time of diagnosis (adjuvant chemotherapy) and 5 years after diagnosis (extended endocrine therapy). These treatment decisions are based on a patient's risk of early and late distant recurrence, respectively. This risk has been traditionally evaluated using standard clinical measures, such as tumor grade, tumor size, age, and Ki67 status. The advent of prognostic molecular assays has improved the accuracy of risk assessment at diagnosis compared with clinical measures only; however, there are limited data regarding the use of prognostic molecular assays for women with node-positive disease and for late recurrence. Validation of these molecular assays for risk of late recurrence and in women with node-positive disease is necessary to evaluate their utility to inform treatment decisions regarding adjuvant chemotherapy and extended endocrine therapy for women with ER-positive, HER2-negative breast cancer.

demonstrated improved survival among women who received endocrine therapy for 10 years versus 5 years (5–8). However, many of these trials are confounded by the inclusion of patients who received adjuvant chemotherapy or by unknown hormone receptor status. In addition, a recent large, randomized, placebo-controlled trial (NSABP-B42) showed that survival was not significantly improved among women with ER-positive, HER2-negative disease who received 10 years of endocrine therapy compared with 5 years (9). Another randomized study of patients who received 5 years of endocrine therapy (ABCSG-16) showed that 5 years of extended anastrozole therapy resulted in added toxicity and did not improve disease-free survival compared with 2 years of extended therapy (10).

In light of this conflicting data regarding the clinical benefits of extended endocrine therapy, treatment decisions at the 5-year time point are not straightforward. There is growing consensus that some patients may be at low enough risk to safely avoid extended endocrine therapy, with recent efforts aimed at identifying reliable prognostic factors. To this end, recently a nomogram was developed to combine nodal status, tumor size, tumor grade, and age (Clinical Treatment Score post-5 years, CTS5) that was significantly prognostic for 5- to 10-year recurrence in women who received 5 years of endocrine therapy (11). However, the authors called out that additional work is needed to investigate this clinicopathologic tool relative to and in combination with molecular assays. With no established prognostic factors for this patient population, professional society guidelines continue to state that all women may "consider" extending endocrine therapy after 5 years and provide no clear guidance on which patients may safely avoid such treatment (1).

With the growing number and complexity of treatment decisions for women with ER-positive, HER2-negative breast cancer, there is an increased need for versatile and reliable prognostic assays. For example, several assays have been validated to predict the risk of 10-year distant recurrence (3, 4). Although this supports the use of prognostic molecular assays in informing initial treatment decisions, additional studies are needed to support their use in clinical decision making for patients with node-positive disease. In addition, a recent study highlighted the long-term risk of recurrence and death from breast cancer, with a 13% risk of recurrence 5 to 20 years after diagnosis for women with node-negative disease who received 5 years of endocrine therapy (12). Although some assays have been shown to be strong predictors of late recurrence 5 to 10 years after diagnosis (13–17), additional studies with longer term follow-up are necessary to more robustly evaluate the use of these molecular assays for extended endocrine therapy decision making.

Previous studies have reported on the use of a 12-gene expression assay in the ABCSG-6 and ABCSG-8 trial cohorts—phase III randomized prospective studies assessing treatment with 5 years endocrine therapy for women with ER-positive, HER2-negative node-negative, and node-positive disease. This included a validation of the prognostic power of the 12-gene assay to predict 10-year distant recurrence (18). In addition, evaluation of the combined ABCSG-6/8 cohort with a median follow-up of 5.3 years showed that the 12-gene assay predicted both early (0–5 years) and late (5–10 years) distant recurrence (14). Up to 15 years of follow-up data are now available for the ABCSG-8 cohort, which enables additional evaluation of the 12-gene assay in light of the current treatment decision dilemmas.

In this study, we reassessed the 12-gene molecular assay in the ABCSG-6/8 combined cohort with longer-term follow-up. This included an assessment of distant recurrence-free rate (DRFR) for newly diagnosed patients, including the subset of patients with node-positive disease. In addition, the risk of late recurrence in the 5- to 15-year time period after diagnosis was evaluated.

Patients and Methods

Cohort

This analysis included patients with early-stage ER-positive, HER2-negative breast cancer who were enrolled in the randomized phase III ABCSG-6 (tamoxifen-only arm) or ABCSG-8 (tamoxifen-only and tamoxifen+anastrozole arms) trials. Patients in both trials, ABCSG-6 or ABCSG-8, did not receive chemotherapy. All women were postmenopausal and received 5 years of adjuvant endocrine therapy only (19, 20). Endocrine therapy included 5 years of tamoxifen or 2 years of tamoxifen followed by 3 years of anastrozole. Some patients enrolled in the ABCSG-6 and ABCSG-8 trials were also enrolled in extended endocrine therapy trials (ABCSG-6a and ABCSG-16; refs. 10, 21). Women who received extended endocrine therapy beyond 5 years were censored at the time of randomization. All patients with ER-positive, HER2-negative breast cancer who had sufficient tumor samples available and successful molecular testing were included, as described previously (18). A total of 1,702 patients met these criteria (378 of 996 women from the tamoxifen-only arm of ABCSG-6; 1,324 of 3,714 women from ABCSG-8). The selection of specimen for this study was previously described by Filipits and colleagues (18).

Gene expression assay

The 12-gene expression assay has been previously described in detail (18, 22). In brief, the RNA expression of 8 target genes (*BIRC5*, *DHCR7*, *UBE2C*, *AZGP1*, *IL6ST*, *MGP*, *RBBP8*, *STC2*) and 3 normalization genes (*CALM2*, *OAZ1*, *RPL37A*) was measured by qRT-PCR. Presence of *HBB* DNA was measured to detect contamination by residual genomic DNA. Samples with

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contaminant genomic DNA received additional DNase treatment and were retested.

A 12-gene molecular score was calculated as the linear combination of the normalized target gene expression (18). Tumors with a molecular score <5 were considered low risk for distant recurrence, whereas scores ≥5 were considered high risk. A combined molecular and clinical score (EPclin score) was calculated by combining the 12-gene molecular score with tumor size and the number of positive lymph nodes (18, 23). Tumors with an EPclin score <3.3 were considered low risk for distant recurrence and tumors with scores ≥3.3 were considered high risk for distant recurrence (18, 24, 25).

Statistical analysis

Primary outcome was DRFR. Time to distant recurrence was defined as the time from randomization to distant recurrence of breast cancer. Documented death due to breast cancer without confirmed distant recurrence was a censoring event (not considered a distant recurrence event). Univariate and multivariable Cox proportional hazard models were used to evaluate the prognostic value of the continuous and categorical (high vs. low risk) 12-gene molecular score and EPclin score on the risk of distant recurrence. Hazard ratios (HR) per unit score were reported. Multivariable analysis adjusted for patient and disease characteristics, including tumor size, nodal status, age, tumor grade, Ki67 expression, ER expression, progesterone receptor expression, and treatment. Multivariable analysis for EPclin score did not include tumor size and nodal status as these variables are part of the EPclin score. Kaplan–Meier estimators were used to estimate DRFR according EPclin risk category and were compared using log-rank test. Analyses were performed for the overall cohort, by nodal status, and for patients who were distant recurrence-free at year 5 (late recurrence). Patient follow-up was not censored for these analyses.

The CTS5 score was calculated as described previously (11). We tested the added prognostic value of EPclin over CTS5, and vice-versa, with likelihood ratio tests applied to multivariable Cox proportional hazard models. Late recurrence was evaluated for the 5- to 15-year period and the 5- to 10-year period, to match the previous validation of the CTS5 score (11).

Statistical analyses were performed using SAS software (SAS Institute Inc.) and R software (R Foundation for Statistical Computing).

Results

Cohort

Maximum follow-up time for this cohort was 16.6 years, with a median of 9.6 years (Table 1). This represents an increase of 4.2 years over previous reports on the combined ABCSG-6/8 cohort (14, 26). In the full cohort, 62.6% (1,066/1,702) of patients had low-risk EPclin scores and 37.4% (636/1,702) of patients had high-risk EPclin scores (Table 1). Baseline clinical characteristics are summarized in Table 1. There were no substantial differences in median age or follow-up time according to molecular score (Supplementary Table S1) or EPclin risk category (Table 1).

Overall, 68.5% (1,166/1,702) of women had node-negative disease and 31.5% (536/1,702) of women had node-positive disease (Table 1). Among women with node-negative disease, 77.8% (907/1,166) had low EPclin scores and 22.2% (259/1,166) had high EPclin scores. Among women with node-positive disease, 29.7% (159/536) had low EPclin scores and 70.3% (377/536) had high EPclin scores. The majority (85.1%) of women with node-positive disease had 1–3 positive lymph nodes, 34.9% (158/453) of whom had low EPclin scores.

Table 1. Patient demographics and clinical characteristics at baseline, according to EPclin risk category

Characteristic	Statistic/category	Low-risk EPclin score ($N = 1,066$)	High-risk EPclin score ($N = 636$)	Total ($N = 1,702$)
Age (years)	Median (IQR)	63 (58–70)	63 (57–71)	63 (58–70)
	Range	41–80	45–79	41–80
Follow-up time[a] (years)	Median (IQR)	9.5 (5.9–11.7)	9.8 (6.1–12.0)	9.6 (6.0–11.9)
	Range	0.0–16.5	0.0–16.6	0.0–16.6
Tumor size	≤1 cm	254 (23.8%)	38 (6.0%)	292 (17.2%)
	1 cm–2 cm	593 (55.6%)	251 (39.5%)	844 (49.6%)
	2 cm–5 cm	213 (20.0%)	326 (51.3%)	539 (31.7%)
	>5 cm	6 (0.6%)	21 (3.3%)	27 (1.6%)
Nodal status	Negative	907 (85.1%)	259 (40.7%)	1166 (68.5%)
	Positive	159 (14.9%)	377 (59.3%)	536 (31.5%)
	1–3 Positive nodes	158 (14.8%)	295 (46.4%)	453 (26.6%)
	≥4 Positive nodes	1 (0.1%)	82 (12.9%)	83 (4.9%)
Tumor grade	Grade 1	283 (26.5%)	78 (12.3%)	361 (21.2%)
	Grade 2	691 (64.8%)	469 (73.7%)	1160 (68.2%)
	Grade 3	11 (1.0%)	50 (7.9%)	61 (3.6%)
	Unknown	81 (7.6%)	39 (6.1%)	120 (7.1%)

Abbreviation: IQR, interquartile range.
[a]Calculated using inverse Kaplan–Meier.

Performance of EPclin in women with newly diagnosed ER$^+$, HER2$^-$ breast cancer

The estimated 10-year DRFR was 95.5% (95% CI, 94.1%–97.0%) for patients with low EPclin scores in the full cohort. In comparison, estimated 10-year DRFR was 80.3% (95% CI, 76.9%–83.9%) for patients with high EPclin scores. This represents a significantly increased risk of distant recurrence among newly diagnosed patients with high-risk EPclin scores (HR 4.77; 95% CI, 3.37–6.67; $P < 0.0001$; Fig. 1A).

In univariate analysis, the 12-gene molecular score was a significant predictor of distant recurrence in the full cohort as a continuous variable (HR 1.28; 95% CI, 1.21–1.35; $P < 0.0001$) and as a categorical variable (HR 2.59; 95% CI, 1.83–3.66; $P < 0.0001$; Supplementary Table S2). EPclin score, which incorporates tumor size and nodal status, was also significant. The performance of the continuous EPclin score (HR 2.66; 95% CI, 2.29–3.09; $P < 0.0001$) and categorical score (HR 4.77; 95% CI, 3.37–6.75; $P < 0.0001$) was improved relative to the molecular score alone (Supplementary Table S2). Tumor size, nodal status, tumor grade, and Ki67 were also significant predictors of distant recurrence in univariate analysis of the full cohort.

Multivariable analysis demonstrated that molecular score remained a significant predictor of distant recurrence after adjusting for clinical factors (HR 1.17; 95% CI, 1.09–1.26; $P < 0.0001$; Supplementary Table S3). This shows that the prognostic information provided by the molecular score is independent of clinical variables. Nodal status, tumor size, age, and Ki67 were also significant (Supplementary Table S3). Multivariable analysis including the EPclin score was performed separately due to the inclusion of nodal status and tumor size in the EPclin score. After

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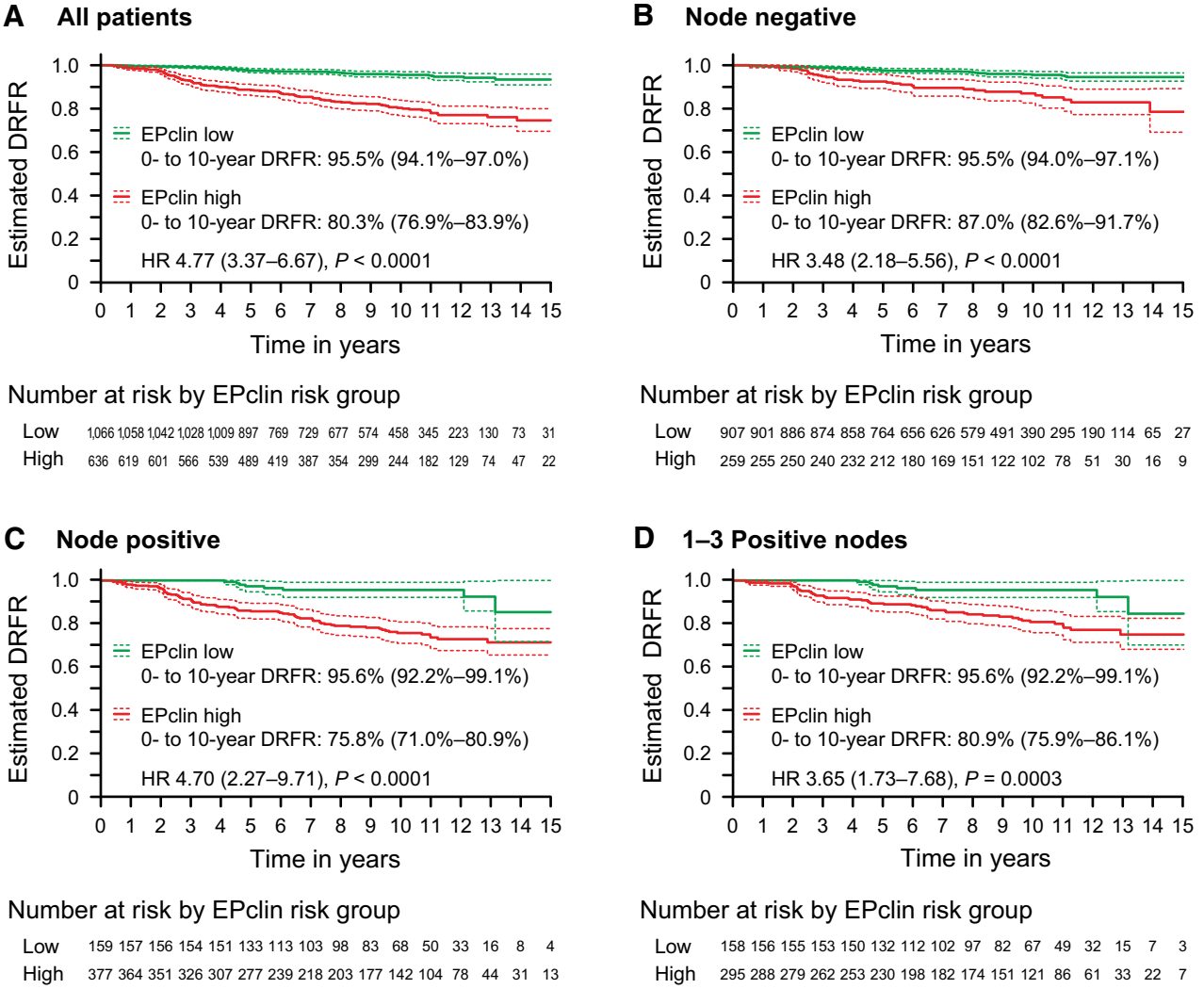

Figure 1.

Kaplan–Meier curves of estimated DRFR for patients with newly diagnosed disease in all patients (**A**), patients with node-negative disease (**B**), all patients with node-positive disease (**C**), and patients with 1 to 3 positive nodes (**D**). DRFR at year 10 (0- to 10-year DRFR) and 95% CIs are provided according to EPclin risk category. HRs and 95% CIs are provided for the likelihood of distant recurrence among patients with high-risk EPclin scores relative to low-risk EPclin scores.

adjusting for other clinical factors, EPclin score (HR 2.55; 95% CI, 2.11–3.08; $P < 0.0001$) remained a significant predictor of distant recurrence (Table 2).

Patients were evaluated separately according to nodal status. Among patients with node-negative disease, estimated 10-year DRFR was 95.5% (95% CI, 94.0%–97.1%) for those with low EPclin scores and 87.0% (95% CI 82.6%–91.7%) for those with high EPclin scores (Fig. 1B). This corresponds with a significantly reduced risk of distant recurrence among patients with low scores (HR 3.48; 95% CI, 2.18–5.56; $P < 0.0001$; Fig. 1B). In univariate analysis, molecular score, EPclin score, tumor size, tumor grade, PR, and Ki67 were significant predictors of distant recurrence among patients with node-negative disease (Supplementary Table S2). Multivariable analysis showed that the EPclin score remained a significant predictor of distant recurrence after adjusting for clinical variables in this population (HR 1.68; 95% CI, 1.18–2.37; $P = 0.0043$; Table 2).

The subset of patients with 1 to 3 positive lymph nodes were evaluated separately. The estimated 10-year DRFR in this subgroup was 95.6% (95% CI, 92.2%–99.1%) for patients with low EPclin scores and 80.9% (95% CI, 75.9%–86.1%) for patients with high EPclin scores (Fig. 1D). This represents a significantly reduced risk of distant recurrence among patients with low scores (HR 3.65; 95% CI, 1.73–7.68; $P = 0.0003$; Fig. 1D). Similar to patients with node-negative disease, molecular score, EPclin score, tumor size, tumor grade, and Ki67 were significant predictors of distant recurrence in univariate analysis among patients with 1 to 3 positive lymph nodes (Supplementary Table S2). In multivariable analysis, the molecular score (HR 1.24; 95% CI, 1.09–1.42; $P = 0.0022$; Supplementary Table S3) and EPclin score (HR 2.68; 95% CI, 1.77–4.08; $P < 0.0001$; Table 2) remained significant predictors of distant recurrence in patients with 1 to 3 positive lymph nodes. Results for all patients with node-positive disease were

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Table 2. Multivariable Cox model with EPclin score for distant recurrence in newly diagnosed patients, according to nodal status

	All patients ($N = 1,702$)		Node-negative ($N = 1,166$)		1-3 Positive nodes ($N = 453$)	
Characteristics	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
EPclin[a]	2.55 (2.11–3.08)	<0.0001	1.68 (1.18–2.37)	0.0043	2.68 (1.77–4.08)	<0.0001
Tumor grade						
Grade 1	Reference	0.1944	Reference	0.3684	Reference	0.0076
Grade 2	1.16 (0.71–1.90)		1.20 (0.62–2.32)		1.75 (0.62–4.97)	
Grade 3	0.64 (0.27–1.50)		2.33 (0.76–7.09)		-	
Age	1.02 (1.00–1.04)	0.0361	1.02 (0.99–1.06)	0.1820	1.01 (0.98–1.04)	0.5424
Ki67	1.01 (0.99–1.02)	0.4666	1.01 (0.98–1.04)	0.4038	1.01 (0.98–1.03)	0.4656
ER						
10%–50%	Reference	0.8934	Reference	0.9358	Reference	0.1788
51%–80%	1.00 (0.58–1.74)		1.01 (0.44–2.35)		0.52 (0.22–1.23)	
81%–100%	0.92 (0.54–1.56)		0.92 (0.41–2.03)		0.43 (0.19–0.99)	
PR						
0%–9%	Reference	0.5964	Reference	0.1359	Reference	0.9382
10%–50%	0.94 (0.59–1.51)		1.12 (0.56–2.23)		0.78 (0.33–1.80)	
51%–80%	0.74 (0.47–1.17)		0.53 (0.25–1.11)		0.85 (0.41–1.72)	
81%–100%	0.91 (0.58–1.44)		1.11 (0.55–2.22)		0.85 (0.40–1.81)	
Treatment						
Tamoxifen + anastrozole	Reference	0.2762	Reference	0.5369	Reference	0.8351
Tamoxifen only	1.22 (0.85–1.74)		1.18 (0.69–2.03)		1.06 (0.61–1.84)	

Abbreviation: PR, progesterone receptor.

[a]HR per unit score after adjusting for age, tumor grade, Ki67, ER, PR, and treatment. Nodal status and tumor size are not included because these variables are included in the EPclin score. Patient follow-up was not censored for this analysis.

similar to patients with 1 to 3 positive nodes (Fig. 1C; Supplementary Tables S2–S4).

Long-term prognostic value of EPclin in women who are distant recurrence-free at 5 years

Overall, 1,386 women were distant recurrence-free at 5 years. Supplementary Fig. S1 shows the relationship between the EPclin score and 5- to 15-year risk of distant recurrence. The estimated DRFR from 5 to 15 years was 95.7% (95% CI, 93.4%–98.1%) for patients who had low EPclin scores and 84.1% (95% CI, 78.9%–89.6%) for patients with high EPclin scores (Fig. 2A). Among women whose disease had not recurred by 5 years, the risk of distant recurrence was significantly reduced among those with low EPclin scores (HR 4.52; 95% CI, 2.65–7.72; $P < 0.0001$; Fig. 2A).

Among women with node-negative disease, the risk of 5- to 15-year distant recurrence was also significantly reduced risk among those with low EPclin scores (HR 3.77; 95% CI, 1.84–7.72; $P < 0.0001$; Fig. 2B). Similar results were observed for women with node-positive disease (HR 3.59; 95% CI, 1.27–10.18; $P = 0.0100$; Fig. 2C) as well as the subset of women with 1 to 3 positive nodes (HR 3.00; 95% CI, 1.03–8.71; $P = 0.0337$; Fig. 2D). Of note is that the number of evaluable patients at the 15-year time point is relatively low, with only 31 evaluable patients with low EPclin scores and 22 with high EPclin scores in the full cohort. This decreases further when nodal status is considered, with only 3 evaluable patients with 1 to 3 positive nodes and low EPclin scores. The resulting confidence intervals for 5- to 15-year survival in patient subgroups according to nodal status are wide due to the low power at this late time point (Fig. 2B–D). However, 5- to 10-year DRFR in all patient subgroups were better powered, with narrow confidence intervals for late recurrence in this time period (Fig. 2; Supplementary Table S5).

Multivariable analysis demonstrated that the molecular score together with tumor size and nodal status remained significant predictors of distant recurrence 5 to 15 years after diagnosis (HR 1.22; 95% CI, 1.08–1.36; $P = 0.0013$; Supplementary Table S6). Multivariable analysis that included the EPclin score demonstrated that only EPclin score remained a significant predictor of distant recurrence in the 5- to 15-year time period after adjusting for clinical variables (HR 2.56; 95% CI, 1.88–3.49; $P < 0.0001$; Table 3). The molecular score (Supplementary Table S5) and EPclin score (Table 3) remained significant predictors of 5- to 15-year distant regardless of nodal status.

EPclin score was evaluated relative to the CTS5 score as a predictor of late recurrence (5- to 15-year period). In the full cohort, the EPclin score significantly added prognostic information to the CTS5 score ($P < 0.001$; Table 4). Conversely, the CTS5 score did not significantly add prognostic information to the EPclin score ($P = 0.0622$). This was also observed for the subset of patients with node-negative disease and node-positive disease (Table 4). Similar results were obtained for the 5- to 10-year time period (Supplementary Table S7).

Discussion

Women with ER-positive, HER2-negative breast cancer are now faced with treatment decisions at 2 different time points—adjuvant chemotherapy at the time of diagnosis and extended endocrine therapy at the 5-year mark. Previous studies have shown that a 12-gene expression assay is prognostic among women with newly diagnosed disease, for both early (0–5 years) and late (5–10 years) distant recurrence (13, 14, 18). However, with more recent data showing that late recurrence can occur well beyond the 10-year time point, an evaluation of prognostic tools with longer term follow-up is relevant to contemporary treatment decisions. The data presented here further affirms EndoPredict as a prognostic tool with longer-term follow-up up to 16 years after diagnosis in women neither treated with adjuvant chemotherapy nor prolonged endocrine therapy.

Of particular note is the prognostic value among women with node-positive disease. About 30% of women with node-positive disease were at low risk for distant recurrence according to the EPclin score. This increased to about 35% when only those with 1 to 3 positive nodes were considered. Importantly, newly diagnosed women with 1 to 3 positive nodes and low-risk EPclin

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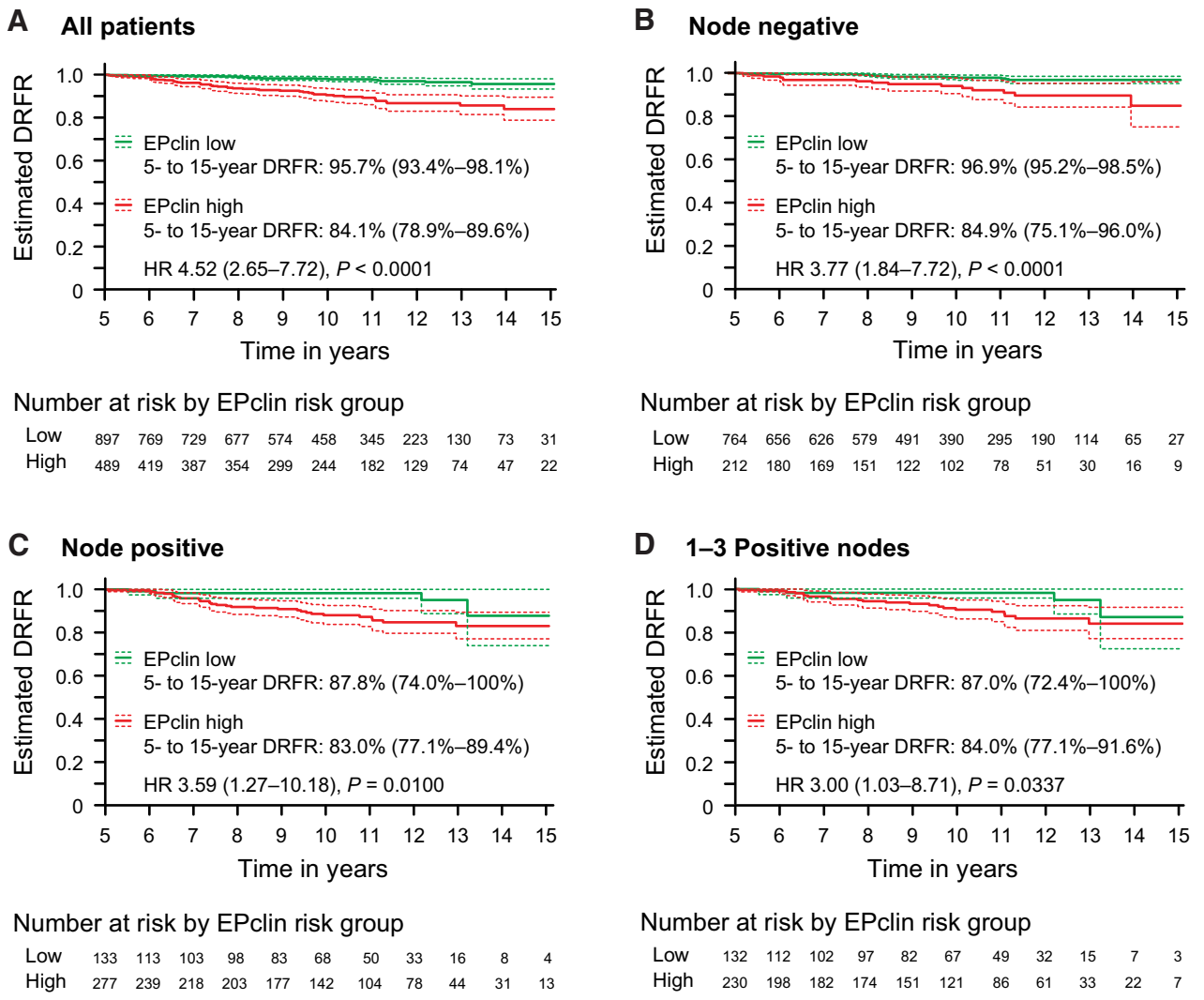

Figure 2.

Kaplan–Meier curves of estimated DRFR for patients who were distant recurrence-free at 5 years for all patients (**A**), patients with node-negative disease (**B**), all patients with node-positive disease (**C**), and patients with 1 to 3 positive nodes (**D**). DRFR at year 15 (5- to 15-year DRFR) and 95% CIs are provided according to EPclin risk category. HRs and 95% CIs are provided for the likelihood of distant recurrence among patients with high-risk EPclin scores relative to low-risk EPclin scores.

scores had an estimated 10-year DRFR of 95.6%, which was significantly improved over those with high-risk EPclin scores. Despite the well reported utility of prognostic assays in women with ER-positive, HER2-negative breast cancer, the limited data available for women with node-positive disease has prevented their widespread use in this population. However, the data presented here demonstrate that the 12-gene assay identifies a population of women with node-positive disease who are at low enough risk for distant recurrence at the time of diagnosis that they may be adequately treated with only 5 years of adjuvant endocrine therapy. This added prognostic information may, therefore, reduce overtreatment in a population of patients who would traditionally be considered high risk for distant recurrence based on positive nodal status alone. These results are consistent with data from the TransATAC trial in which patients with 1 to 3 positive lymph nodes and EPclin low-risk classification had a DRFR of 94.4% (13). Collectively, this provides strong evidence of the prognostic value of EPclin in the subgroup of patients with node-positive disease (27).

The analysis of the ABCSG-6/8 cohorts with this longer-term follow-up also enabled an evaluation of the prognostic value of the 12-gene assay in women who were distant recurrence-free 5 years after diagnosis. Previous studies have demonstrated that the EPclin score is a significant predictor of late DRFR from 5 to 10 years (13, 14). However, the data presented here showed that women with low EPclin scores had significantly improved DRFR even in the longer follow-up period from 5 to 15 years compared with those with high EPclin scores. After adjusting for clinical variables, EPclin score remained a significant predictor of late distant recurrence. Patients without recurrence after 5 years of endocrine therapy and a low-risk classification by EPclin had an estimated DRFR of 95.7% without extension of endocrine

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Published OnlineFirst May 7, 2019; DOI: 10.1158/1078-0432.CCR-19-0376

Table 3. Multivariable Cox model with EPclin score for late recurrence in patients who were distant recurrence-free after 5 years, according to nodal status

	All patients ($N = 1{,}386$)		Node-negative ($N = 975$)		1–3 Positive nodes ($N = 362$)	
Characteristic	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
EPclin[a]	2.56 (1.88–3.49)	<0.0001	2.01 (1.19–3.39)	0.0101	3.43 (1.74–6.76)	0.0005
Tumor grade						
Grade 1	Reference	0.1313	Reference	0.5087	Reference	0.0826
Grade 2	1.33 (0.61–2.88)		1.84 (0.62–5.51)		1.38 (0.31–6.16)	
Grade 3	0.38 (0.07–2.04)		1.51 (0.16–14.67)		—	
Age	1.00 (0.96–1.03)	0.8918	1.00 (0.95–1.05)	0.9003	0.99 (0.94–1.04)	0.7528
Ki67	1.01 (0.98–1.03)	0.7192	1.01 (0.97–1.05)	0.6049	1.01 (0.96–1.05)	0.8082
ER						
10%–50%	Reference	0.3955	Reference	0.5258	Reference	0.5842
51%–80%	1.61 (0.58–4.46)		1.96 (0.40–9.57)		0.46 (0.11–1.91)	
81%–100%	1.86 (0.71–4.84)		2.23 (0.49–10.14)		0.50 (0.13–1.97)	
PR						
0%–9%	Reference	0.6230	Reference	0.4505	Reference	0.7023
10%–50%	0.92 (0.42–2.03)		0.57 (0.17–1.85)		1.46 (0.36–5.81)	
51%–80%	0.78 (0.38–1.62)		0.54 (0.19–1.51)		1.80 (0.52–6.18)	
81%–100%	1.23 (0.60–2.52)		1.07 (0.39–2.89)		2.10 (0.58–7.66)	
Treatment						
Tamoxifen + anastrozole	Reference	0.9280	Reference	0.8997	Reference	0.2279
Tamoxifen only	0.98 (0.57–1.66)		0.95 (0.44–2.07)		0.60 (0.26–1.37)	

Abbreviation: PR, progesterone receptor.

[a]HR per unit score after adjusting for age, tumor grade, Ki67, ER, PR, and treatment. Nodal status and tumor size are not included because these variables are included in the EPclin score. Patient follow-up was not censored for this analysis.

therapy. As such, EPclin may aid in the identification of patients at such low risk of late recurrence from 5 to 15 years that they may safely avoid extended endocrine therapy.

A limitation of this study is the number of patients with follow-up data beyond 12 years. Although the maximum follow-up time was over 15 years, there were very few evaluable patients at the 15-year time point. As such, Kaplan–Meier survival analysis was limited in power for some patient subgroups. For example, the 5- to 15-year DRFR for patients with node-positive disease was similar for low-risk (87.0%) and high-risk patients (84.0%). This is driven by the limited power at 15 years for this subgroup. However, DRFR curves for high- and low-risk patients with node-positive disease are significantly different at earlier time points. This is consistent with existing data on late-recurrence from 5 to 10 years and may still support different treatment decisions for high- and low-risk patients. In addition, the longer-term follow-up data presented here was sufficiently powered to assess the value of a prognostic assay in the 5- to 15-year time frame for the first time in the whole cohort of patients.

In summary, the data presented here demonstrate the prognostic value of the EPclin score in predicting early and late distant recurrence among women ER-positive, HER2-negative breast cancer. The EPclin score was a significant predictor of DRFR among women with newly diagnosed disease, which may aid in identifying patients having most likely no additional benefit from adjuvant chemotherapy. This is of particular importance for women with 1 to 3 positive nodes who have low-risk disease and would likely receive chemotherapy without the added prognostic information obtained from EPclin. In addition, there is significant prognostic value in predicting 15-year distant recurrence among women who are distant recurrence-free at 5 years and are faced with the decision of whether or not to extend endocrine therapy. Collectively, this demonstrates the prognostic value of the 12-gene molecular assay in making treatment decisions for women with ER-positive, HER2-negative breast cancer.

Table 4. Likelihood ratio analysis of CTS5 and EPclin for 5- to 15-year DRFR

Patients	*N*	Model 1	Model 2	LR Chi square	*P* value
All patients	1,284	CTS5	CTS5 + EPclin	16.0	<0.0001
		EPclin	EPclin + CTS5	3.5	0.0622
Node negative	913	CTS5	CTS5 + EPclin	7.7	0.0055
		EPclin	EPclin + CTS5	1.3	0.2486
Node positive	371	CTS5	CTS5 + EPclin	7.9	0.0048
		EPclin	EPclin + CTS5	0.8	0.3627

Abbreviations: CTS5, clinical treatment score after 5 years; LR, likelihood ratio.

Disclosure of Potential Conflicts of Interest

M. Filipits reports receiving commercial research grants from Myriad Genetics, Inc. and reports receiving other remuneration from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme, Myriad Genetics, Inc., Novartis, Ratiopharm, and Roche. Z. Bago-Horvath reports receiving speakers bureau honoraria from Roche, and is a consultant/advisory board member for Roche and Biomedica. C.F. Singer reports receiving speakers bureau honoraria from Novartis and AstraZeneca, and is a consultant/advisory board member for Roche, Novartis, Genomic Health, and Pfizer. K. Brown is an employee of Myriad Genetics, Inc. R. Bernhisel is an employee of Myriad Genetics, Inc. R. Kronenwett is an employee of Myriad International GmbH, previously held ownership interest (including patents) in Milestone Payment, and is listed as a co-inventor on a patent application that claims that the prognostic gene expression test EndoPredict, which is used in the study, can be used to predict benefit from chemotherapy, to be owned by Myriad Genetics. J.M. Lancaster is an employee of and holds ownership interest (including patents) in Myriad Genetics, Inc., and is a consultant/advisory board member for Protean Biodiagnostics. F. Fitzal holds ownership interest (including patents) in and is a consultant/advisory board member for Pfizer, Novartis, AstraZeneca, Roche, Lilly, COmesa, and Bondimed. M. Gnant has immediate family members employed by Sandoz, and reports receiving speakers bureau honoraria from Celgene, Roche/Genentech, Novartis, AstraZeneca, Amgen, Nanostring, Eli Lilly, Pfizer, Medison, Ipsen, and Medtronic. No potential conflicts of interest were disclosed by the other authors.

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Published OnlineFirst May 7, 2019; DOI: 10.1158/1078-0432.CCR-19-0376

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Acknowledgments

This work was supported by the Myriad Genetics, Inc.

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Received January 30, 2019; revised April 9, 2019; accepted May 2, 2019; published first May 7, 2019.

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Published OnlineFirst May 7, 2019; DOI: 10.1158/1078-0432.CCR-19-0376

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Prediction of Distant Recurrence Using EndoPredict Among Women with ER $^{+}$, HER2^{-} Node-Positive and Node-Negative Breast Cancer Treated with Endocrine Therapy Only

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Clin Cancer Res Published OnlineFirst May 7, 2019.

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