Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Breast Cancer Index Identifies Early-Stage Estrogen Receptor–Positive Breast Cancer Patients at Risk for Early- and Late-Distant Recurrence

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Abstract

Purpose: Residual risk of relapse remains a substantial concern for patients with hormone receptor–positive breast cancer, with approximately half of all disease recurrences occurring after five years of adjuvant antiestrogen therapy.

Experimental Design: The objective of this study was to examine the prognostic performance of an optimized model of Breast Cancer Index (BCI), an algorithmic gene expression–based signature, for prediction of early (0–5 years) and late (>5 years) risk of distant recurrence in patients with estrogen receptor–positive (ER^+), lymph node–negative (LN^-) tumors. The BCI model was validated by retrospective analyses of tumor samples from tamoxifen-treated patients from a randomized prospective trial (Stockholm TAM, $n = 317$) and a multi-institutional cohort ($n = 358$).

Results: Within the Stockholm TAM cohort, BCI risk groups stratified the majority (~65%) of patients as low risk with less than 3% distant recurrence rate for 0 to 5 years and 5 to 10 years. In the multi-institutional cohort, which had larger tumors, 55% of patients were classified as BCI low risk with less than 5% distant recurrence rate for 0 to 5 years and 5 to 10 years. For both cohorts, continuous BCI was the most significant prognostic factor beyond standard clinicopathologic factors for 0 to 5 years and more than five years.

Conclusions: The prognostic sustainability of BCI to assess early- and late-distant recurrence risk at diagnosis has clinical use for decisions of chemotherapy at diagnosis and for decisions for extended adjuvant endocrine therapy beyond five years. *Clin Cancer Res; 19(15); 4196–205. ©2013 AACR.*

Introduction

Breast cancer is a heterogeneous disease with a significant proportion of patients with breast cancer diagnosed with estrogen receptor–positive (ER^+) tumors with lymph node–negative (LN^-) status at diagnosis (1). Compared with other clinical subgroups, ER^+ LN^- patients have the best overall prognosis. However, the rate of recurrence for ER^+ LN^- patients surpasses those of other clinical subgroups [triple-negative and HER2–positive ($HER2^+$)] for patients that remain disease-free for 5 years (2). For ER^+ LN^- patients, more than half of recurrences occur beyond 5 years after primary treatment (3), and the annual rate of recurrence remains substantial in later years (5.2% annually between years 5 and 8 and 4.6% annually between years 8 and 12; ref. 4). Comprehensive assessment of risk of recurrence for ER^+ LN^- patients with a time horizon that spans from diagnosis to 10 years would be clinically useful for identifying both patients with sustained low risk of recurrence for avoidance of chemotherapy and those with high risk of recurrence beyond 5 years for the decision of whether to extend adjuvant endocrine therapy. In current clinical practice, physicians use clinicopathologic parameters (e.g., Adjuvant! Online) and gene expression–based tests (e.g., the 21-gene assay) to assess recurrence risk for 0 to 10 years. However, the predictive performance of both methodologies is mostly limited to the first 5 years (5–8). Given the persistent risk of recurrence beyond 5 years, there is a significant clinical unmet need for biomarkers that accurately assess the risk of recurrence across the full disease time horizon.

Breast Cancer Index (BCI) is a gene expression–based biomarker that was developed using a cohort of tamoxifen-treated patients from the randomized prospective Stockholm trial and has been shown to provide an individual risk of distant breast cancer recurrence based on a continuous risk model (9). BCI was developed through

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Translational Relevance

Late recurrence of hormone receptor–positive breast cancer represents a significant clinical challenge, as more than half of all relapses occur after five years from the time of diagnosis. Current signatures predict for recurrence risk within five years of diagnosis, but biomarkers for prediction of late (>5 years) disease recurrence are lacking. In this study, a linear model of Breast Cancer Index (BCI), an algorithmic gene expression–based signature was evaluated for its ability to predict both early (0–5 years) and late risk of distant recurrence in estrogen receptor–positive (ER^+) lymph node–negative (LN^-) breast cancer. This research has direct application to patient care, as the use of such a biomarker could enable clinicians to more clearly assess recurrence risk within a time continuum that is aligned with the disease horizon of hormone receptor–positive breast cancer, and would identify patients that may or may not require extended adjuvant endocrine therapy.

the algorithmic combination of 2 previously described biomarkers, *HOXB13:IL17BR* ratio (H/I) and the Molecular Grade Index (MGI), and provided superior prognostic performance than either biomarker alone (10–12). In previous studies, BCI has been shown to significantly predict 0- to 10-year risk of recurrence beyond standard clinicopathologic factors (9, 13).

The objective of this study was to develop an optimized BCI risk model and validate its prognostic performance with prespecified risk groups for predicting early (0–5 years) and late (>5 years) distant recurrences. The optimized model was developed using a cohort of untreated patients from the randomized prospective Stockholm trial to better represent the natural history of breast cancer recurrence. This BCI model was then validated in retrospective analyses of two cohorts: first, tumor samples from tamoxifen-treated ER^+ LN^- patients enrolled in the Stockholm trial (Stockholm TAM; ref. 14) and second, a multi-institutional cohort consisting of ER^+ LN^- tamoxifen-treated patients from 2 academic medical centers (12, 13).

Materials and Methods

Patients and tumor samples

The Stockholm study was a prospective, randomized trial during 1976 through 1990 comparing 2 or 5 years of adjuvant tamoxifen versus untreated in 2,738 postmenopausal women with invasive early-stage breast cancer, including 1,780 low-risk LN^- patients with tumor size 30 mm or less in diameter (14). The benefit from tamoxifen was largely independent of duration of the therapy in the Stockholm study. For this study, formalin-fixed paraffin-embedded (FFPE) tumor blocks from 808 LN^- patients were received. As tumors were not graded during the actual trial, tumor grades were retrospectively determined by one pathologist (D. Sgroi) blinded to outcome according to the Nottingham system. Follow-up data were collected from regional population registers and the Swedish Cause of Death Registry, with median follow-up time of 17 years.

The second cohort used in this study was ER^+ LN^- breast cancer patients identified from University of Pittsburgh Medical Center (UPMC; Pittsburgh, PA) and Massachusetts General Hospital (MGH; Boston, MA) who were diagnosed between 1990 and 2000 with FFPE tumor blocks available. Two hundred and sixty five and 93 specimens were selected from UPMC and MGH, respectively, for this study as previously described (12, 13). Follow-up data were retrieved from the Cancer Registry Data at UPMC and at MGH. A hematoxylin and eosin (H&E) slide for each case was reviewed to confirm tumor grade by the Nottingham system.

The investigation of the collected tumor samples was approved by the Institutional Review Board at each institution. In accordance with the approval, informed consent from patients was not required.

Hormonal receptor status

For the Stockholm cohort, status of ER, progesterone receptor (PR), and HER2 was assessed retrospectively with immunohistochemistry (IHC). The cutoff level for ER and PR positivity was set to 1% positively stained tumor cell nuclei. Because FISH for HER2 confirmation was not conducted for these patients, the final HER2 status for cases with missing HER2 status and those with IHC 2+ was determined by real-time reverse-transcription PCR (RT-PCR) measurement using a cutoff (−0.05) that achieved the greatest accuracy (97% concordance) in a previous cohort for which IHC/FISH testing results were available (data submitted for publication).

For specimens from UPMC and MGH, ER status was determined by IHC through pathology report review with a cutoff level of 10% nuclear staining of tumor cells (clinical practice standard at the time) as a sample inclusion criterion. Although different cutoff levels were used for Stockholm versus UPMC and MGH cohorts, only 8 of 600 ER^+ patients (1.4%) from Stockholm cohort were identified as ER^+ by a 1% cutoff rather than a 10% cutoff. For the analysis in this study, HER2 status was determined using RT-PCR measurement as described earlier.

RT-PCR analysis and calculation of gene expression indices

Gene expression analysis of FFPE specimens was conducted at bioTheranostics, Inc., blinded to clinical outcome. The genes analyzed were *HOXB13*, *IL17BR* (H/I), *BUB1B*, *CENPA*, *NEK2*, *RACGAP1*, *RRM2* (MGI), *ACTB*, *HMBS*, *SDHA*, and *UBC* (reference genes). Primer and probe sequences were previously described (12, 15). For each case, 10-μm tissue sections were cut and an H&E slide was generated to confirm 40% content of invasive cancer. Manual macrodissections were carried out on all sections to enrich tumor content before RNA extraction. Total RNA was reverse transcribed and the resulting cDNA was preamplified by PCR using the PreAmp Master Mix Kit as per the manufacturer's instructions (Applied Biosystems) before being

analyzed by TaqMan RT-PCR as previously described (12). The expression of each gene was normalized relative to the 4 reference genes using the cycle threshold (C_T) values, and these normalized expression values were used to calculate H/I and MGI as previously described (12, 15). Precision/reproducibility of the BCI assay was completed under quality system regulation as part of the clinical laboratory quality management process. The intra-assay reproducibility was evaluated by conducting BCI assays on 6 specimens divided into 4 replicates. The intra-assay coefficient of variation (CV) ranged from 0.19% to 2.23% for the genes assayed. The inter-assay reproducibility was assessed by conducting BCI assays on 9 specimens, each run by three operators repeated at least four independent times across a span of 2 weeks. The inter-assay CV ranged from 0.91% to 1.77% for the genes analyzed. In addition, the inter-assay CV estimated from conducting the BCI assay in 42 independent batches with the universal reference RNA as a positive control during this study ranged from 0.86% to 1.40% for the genes analyzed.

Development of a continuous risk index

Initial development used a continuous risk model with a cubic component built using the tamoxifen-treated arm of the Stockholm trial (BCI-Cubic-TAM; ref. 9). In the current study, an optimized continuous recurrence risk model (BCI) was built by combining H/I and MGI into a continuous index using the ER^+ LN^- patients from the untreated arm of the Stockholm cohort as the training set. Linearity of H/I and MGI was confirmed by likelihood ratio tests with Cox proportional hazard regression models with and without restricted cubic splines. The raw BCI risk index was produced by a linear combination of H/I and MGI weighed by the corresponding coefficients derived from a Cox regression model including both H/I and MGI as independent variables. The raw index was then rescaled into a final score in the range of 0 to 10. The intra-assay standard deviation (SD) of BCI scores was 0.24 and the interassay SD was 0.41. The BCI was further categorized into three risk groups based on two cutoffs: low risk if BCI < 5.0825, intermediate risk if $5.0825 \leq$ BCI < 6.5025, and high risk if BCI $\geq$ 6.5025. These two cutoffs were chosen to have similar proportions of patients in the three risk groups as stratified by the combination of the binary H/I and MGI risk groups: low (low MGI), intermediate (high MGI and low H/I), and high (high MGI and high H/I) based on previously established and validated cutoffs for each biomarker (9, 12, 15). These cutoffs for BCI risk groups corresponded to the 10-year recurrence risks in the Stockholm untreated patients (training set) of 17.5% and 27.5%, respectively.

Statistical analysis

All BCI analyses of Stockholm TAM and multi-institutional cohorts were completed with a prespecified BCI model and BCI risk groups. The study endpoint as stated in the statistical analysis plan was distant recurrence-free survival defined as the time from diagnosis to the time of first distant metastasis; patients were censored at the time of second primary or death or at the last follow-up. In the multi-institutional cohort, 13 deaths without documented recurrence were censored. Late-distant recurrences referred to those occurring after 5 years from diagnosis and were evaluated within the subset of patients who had remained recurrence free for at least 5 years to assess whether the gene signature still remains prognostic after its prognostic effect during the first 5 years (early recurrence) had been removed. Kaplan–Meier survival analysis was used to graphically present the survival curves of BCI's three prespecified risk groups and the equality of the survival curves was evaluated with log-rank test. Multivariate Cox proportional hazard models were used to evaluate whether BCI, as a continuous risk index provided prognostic information independent of traditional clinical and pathologic factors (age, tumor size, tumor grade, HER2 status, PR status, and chemotherapy if appropriate) using likelihood ratio tests. Considering limited number of events, a stepwise variable selection based on Akaike's Information Criterion (AIC) was used to select simplified multivariate models. HRs and associated 95% confidence intervals (CI) were provided. A P value less than 0.05 (two-sided) was considered statistically significant. All analyses were conducted using R statistical package (version 2.12.2; http://www.r-project.org).

Results

Patient and tumor characteristics

Two patient cohorts were used in this study (Supplementary Fig. S1). For the Stockholm prospective trial cohort, tumor samples from 600 ER^+ LN^- postmenopausal patients were examined with a similar number of patients from both arms: tamoxifen-treated ($n = 317$) and -untreated ($n = 283$) patients. Patient and tumor characteristics are shown in Table 1. Patients from both arms had non-statistical differences in all examined clinicopathologic variables (age, tumor size, and tumor grade) with the majority of patients (~80%) having small tumors (≤20 mm). In addition, biomarker status of HER2 and PR was similar between untreated and tamoxifen-treated patient cohorts. The overall median time of follow-up for all patients was 17 years and the numbers of distant recurrences were 56 (20%) and 33 (10%) for untreated and tamoxifen-treated patients, respectively, with 52% and 61% of the recurrences occurring after 5 years. The second cohort is a multi-institutional cohort of tumor samples of ER^+ LN^- patients of cases from UPMC ($n = 265$ consecutive) and MGH ($n = 93$). As compared with the Stockholm trial, the multi-institutional cohort includes both premenopausal (~30%) and postmenopausal patients, larger tumors (66% T1, 34% T2 and T3), and patients treated with adjuvant chemotherapy (32%; Table 1). The overall median time of follow-up for all patients was 10 years and the rate of distant recurrence was 16% with 40% of the recurrences occurring after 5 years.

Development of BCI as a continuous risk index using the Stockholm trial untreated arm

Individually, MGI and H/I each had significantly prognostic performance in the Stockholm trial untreated cohort. However, MGI showed greater prognostic performance

Table 1. Patient clinicopathologic characteristics in Stockholm and multi-institutional cohorts

	Stockholm untreated ($n = 283$)	Stockholm TAM-treated ($n = 317$)	Multi-institutional ($n = 358$)
Age at surgery, y			
<50	4 (1%)	2 (<1%)	108 (30%)
50–59	92 (33%)	89 (28%)	113 (32%)
60–69	178 (63%)	214 (68%)	93 (26%)
≥70	9 (3%)	12 (4%)	44 (12%)
Tumor size			
≤20 mm	230 (81%)	259 (82%)	237 (66%)
>20 mm	51 (18%)	55 (17%)	121 (34%)
Unknown	2 (1%)	3 (1%)	0
Tumor grade			
Well	69 (24%)	67 (21%)	82 (23%)
Moderate	175 (62%)	211 (67%)	219 (61%)
Poor	39 (14%)	39 (12%)	57 (16%)
PR status			
Negative	73 (26%)	72 (23%)	NA
Positive	182 (64%)	220 (69%)	
Unknown	28 (10%)	25 (8%)	
HER2 status			
Negative	257 (91%)	295 (93%)	316 (88%)
Positive	26 (9%)	22 (7%)	42 (12%)
Adjuvant chemotherapy			
No	283 (100%)	317 (100%)	243 (68%)
Yes	0	0	115 (32%)
Distant recurrence			
Overall	56 (100%)	33 (100%)	57 (100%)
Early (≤5 y)	27 (48%)	13 (39%)	34 (60%)
Late (>5 y)	29 (52%)	20 (61%)	23 (40%)
BCI groups			
Low	156 (55%)	202 (64%)	196 (55%)
Intermediate	75 (27%)	65 (20%)	78 (22%)
High	52 (18%)	50 (16%)	84 (23%)

Abbreviation: NA, not available.

from 0 to 5 years, whereas H/I showed greater prognostic performance beyond 5 years (Supplementary Fig. S2). An optimized continuous risk index (BCI) was built from a linear combination of H/I and MGI using the ER^+ LN^- patients from the untreated arm of the Stockholm cohort as the training set ($n = 283$). On the basis of the predetermined cutoff points, 156 (55%) patients were classified as low risk, 75 (27%) as intermediate risk, and 52 (18%) as high risk with mean 10-year rates of distant recurrence of 11.2% (95% CI, 6.9%–16.2%), 21.0% (95% CI, 10.6%–30.3%), and 34.4% (95% CI, 19.0%–46.9%) respectively. The log-rank test comparing the three BCI risk groups were significant ($P = 0.0001$), with a HR of 2.13 (95% CI, 1.13–4.03) for intermediate versus low risk and a HR of 3.69 (95% CI, 1.95–6.98) for high versus low risk.

Validation of BCI for early- and late-distant recurrence in Stockholm TAM and multi-institutional cohorts

The Stockholm TAM and multi-institutional cohorts were used to validate the performance of BCI to stratify ER^+ LN^- tamoxifen-treated patients for risk of overall (0–10 years), early (0–5 years), and late recurrence (>5 years). BCI was a highly significant prognostic in both cohorts for assessing overall (0–10 years) distant recurrence risk after adjusting for clinicopathologic variables (see Supplementary Tables S1 and S2 and Supplementary Figs. S3 and S4).

BCI was a significant predictor for risk assessment of both early- (0–5 years) and late-distant recurrence (>5 years) in the Stockholm TAM ($n = 317$). For early recurrence, BCI classified 64% patients into low-, 20% into intermediate-, and 16% into high-risk group, with 5-year distant recurrence-free survival at 98.0% (95% CI, 96.0%–100%), 95.2% (95% CI, 90.1%–100%), and 87.8% (95% CI, 79.0%–97.4%), respectively (Table 2). The Kaplan–Meier estimates of likelihood of early-distant recurrence comparing the three BCI risk groups were significant ($P = 0.0063$), with a univariate HR of 2.31 (95% CI, 0.52–10.3) for intermediate versus low risk and a HR of 6.19 (95% CI, 1.75–21.92) for high versus low risk (Fig. 1A). Multivariate Cox regression adjusting for clinicopathologic factors with stepwise variable selection showed that BCI was the only significant predictive risk factor remained for early recurrence (HR, 9.08; 95% CI, 1.86–44.4; $P = 0.006$; Table 3). In ER^+ $HER2^-$ patients ($n = 295$), BCI remained the sole significant predictor retained in multivariate analysis with a HR of 16.25 (95% CI, 2.59–102.0; $P = 0.003$; Table 3) after stepwise variable selection.

Patients within the Stockholm TAM cohort who remained distant recurrence-free for 5 years were examined to validate the performance of BCI to stratify patient risk for late recurrence ($n = 285$). BCI classified 65%, 20%, and 15% patients into low-, intermediate-, and high-risk group with 10-year distant recurrence-free survival at 97.2% (95% CI, 94.8%–99.7%), 92.8% (95% CI, 86.2%–99.9%), and 89.9% (95% CI, 80.9%–99.8%), respectively (Table 2). The log-rank test P value comparing the Kaplan–Meier curves of BCI risk groups was significant ($P = 0.0152$), with a univariate HR of 2.20 (95% CI, 0.72–6.73) for intermediate versus low risk and 4.04 (95% CI, 1.46–11.14) for high versus low risk (Fig. 1B). In multivariate Cox regression including clinicopathologic variables with stepwise variable selection, BCI was the only significant predictive factor with a HR of 3.50 (95% CI, 1.09–11.21; $P = 0.035$; Table 4). In ER^+ $HER2^-$ patients ($n = 266$), BCI remained a significant predictor in the multivariate analysis with HR of 4.57 (95% CI, 1.28–16.37; $P = 0.020$; Table 4) after variable selection. Individual risk of distant recurrence by a continuous BCI score for both 0 to 5 years and more than 5 years increased monotonically as the BCI score increased (Fig. 2A and B). Overall, BCI scores for individual risk assessment of early- and late-distant recurrence (0–5 years, >5 years) were similar in rates of distant recurrence for Stockholm TAM and when combining the two (MGH and UPMC) cohorts (Fig. 2 and Supplementary Fig. S6).

Table 2. Kaplan–Meier estimates of early- and late-distant recurrence-free survival for 3 BCI risk groups in Stockholm TAM and multi-institutional cohorts

	Stockholm TAM-treated			Multi-institutional		
Patient subgroups	**No. of patients (%)**	**No. DR**	**DRFS (95% CI)**	**No. of patients (%)**	**No. DR**	**DRFS (95% CI)**
Early-distant recurrence at 5 y						
BCI low risk	202 (64%)	4	98.0 (96.0–100)	196 (55%)	8	95.9 (93.1–98.7)
BCI intermediate risk	65 (20%)	3	95.2 (90.1–100)	78 (22%)	6	92.3 (86.5–98.4)
BCI high risk	50 (16%)	6	87.8 (79.0–97.4)	84 (23%)	20	75.5 (66.7–85.4)
Late-distant recurrence at 10 y[a]						
BCI low risk	184 (65%)	8	97.2 (94.8–99.7)	181 (58%)	4	97.5 (95.0–100.0)
BCI intermediate risk	58 (20%)	5	92.8 (86.2–99.9)	70 (22%)	10	83.1 (73.8–93.5)
BCI high risk	43 (15%)	7	89.9 (80.9–99.8)	61 (20%)	9	85.0 (76.4–94.5)

Abbreviations: DR, distant recurrence; DRFS, distant recurrence-free survival.

[a]In patients who were recurrence-free at 5 years.

For predictive performance of early-distant recurrence within the multi-institutional cohort ($n = 358$), BCI stratified 55%, 22%, and 23% patients into low-, intermediate-, and high-risk groups, respectively, with 5-year distant recurrence-free survival at 95.9% (95% CI, 93.1%–98.7%), 92.3% (95% CI, 86.5%–98.4%), and 75.5% (95% CI, 66.7%–85.4%), respectively (Table 2). The Kaplan–Meier estimate of likelihood of distant metastasis comparing the three BCI risk groups was highly significant ($P < 0.0001$), with a univariate HR of 1.93 (95% CI, 0.67–5.57) for intermediate versus low risk and 6.55 (95% CI, 2.89–14.88) for high versus low risk (Fig. 1C). Multivariate Cox regression with stepwise variable selection showed that BCI was the only significant predictive risk factor (HR, 11.50; 95% CI, 4.22–31.32; $P < 0.0001$; Table 3). In ER^+ $HER2^-$ patients ($n = 316$), BCI was the only significant predictor retained in multivariate analysis with stepwise variable selection with a HR of 13.71 (95% CI, 4.54–41.36; $P < 0.0001$; Table 3).

Patients within the multi-institutional cohort who remained distant recurrence-free for 5 years were examined to validate the performance of BCI to stratify patient risk for late recurrence ($n = 312$). BCI classified 58%, 22%, and 20% patients into low-, intermediate-, and high-risk group with late-distant recurrence at 10 years of 97.5% (95% CI, 95.0%–100.0%), 83.1% (95% CI, 73.8%–93.5%), and 85.0% (95% CI, 76.4%–94.5%), respectively (Table 2). The log-rank test P value comparing the Kaplan–Meier curves of BCI risk groups was significant ($P = 0.0002$), with a univariate HR of 6.9 (95% CI, 2.17–22.02) for intermediate versus low risk and 7.03 (95% CI, 2.17–22.84) for high versus low risk (Fig. 1D). In multivariate Cox regression with stepwise variable selection, BCI was the most significant predictive factor with a HR of 9.24 (95% CI, 2.85–30.00; $P = 0.0002$; Table 4) along with tumor size being significant (HR, 2.66; 95% CI, 1.14–6.20; $P = 0.02$; Table 4). In ER^+ $HER2^-$ patients ($n = 281$), BCI remained the most significant predictor in multivariate analysis with a HR of 9.33 (95% CI, 2.83–30.76; $P = 0.0002$; Table 4); tumor size was also significant (HR, 2.53; 95% CI, 1.07–5.97; $P = 0.03$; Table 4).

Discussion

BCI has significant sustainable prognostic performance that spans from the time of diagnosis to 10 years after diagnosis for the prediction of individual risk of distant recurrence for ER^+ LN^- tamoxifen-treated patients. Within the Stockholm TAM cohort, BCI risk groups stratified the majority (~65%) of patients as low risk with 2% and 2.8% distant recurrence rates for early (0–5 years) and late recurrence (>5 years), respectively. This indicates that patients classified by BCI as low risk at diagnosis continue to have a low risk of recurrence throughout a 10-year horizon. Conversely, BCI stratified a small proportion (~15%) of patients within the high-risk group with distant recurrence rates more than 10% for both early- and late-distant recurrence. Sustainable BCI low- and high-risk groups for early and late recurrence were also maintained within a multi-institutional cohort. BCI classified a large proportion (55%–58%) of patients as low risk with 4.1% and 2.5% distant recurrence rates for early and late recurrence, and a smaller proportion (20%–23%) of patients as high risk, with 24.5% and 15% distant recurrence rates for early and late recurrence, respectively.

The BCI intermediate-risk group within the multi-institutional cohort has a similar risk profile as BCI low risk for 0 to 5 years (Fig. 1C); however, for more than 5 years, the intermediate and high-risk groups are statistically indistinguishable and both are significantly different from low risk (Fig. 1D). In the Stockholm TAM cohort, the BCI intermediate-risk group shows a similar risk pattern, although the difference for more than 5 years between the intermediate-risk group and the low-risk group did not

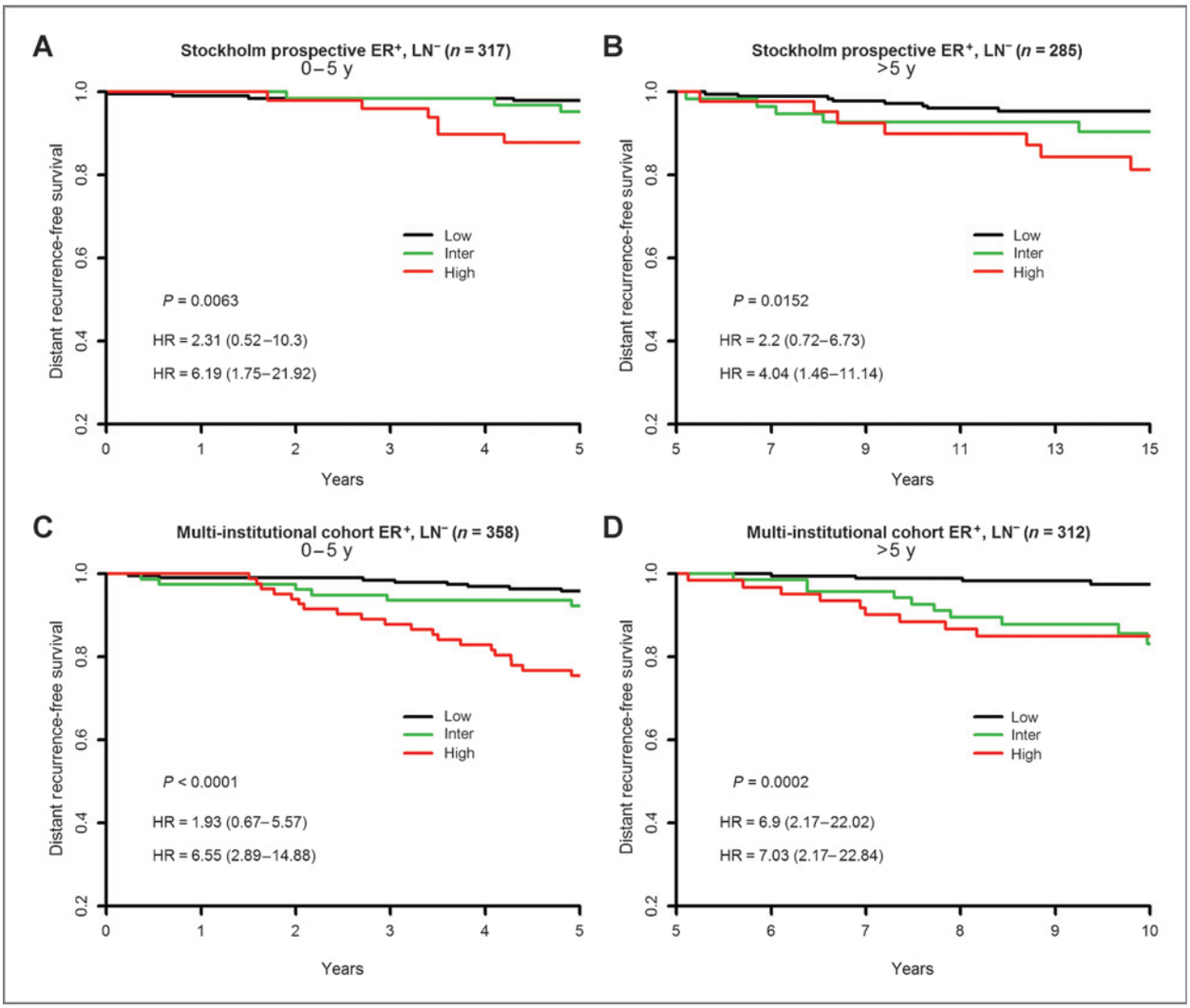

Figure 1. BCI risk groups for prediction of early- and late-distant recurrence in the Stockholm TAM cohort (ER^+, LN^-) and the multi-institutional cohort (ER^+ LN^-). A and C, early-distant recurrence (0–5 years). B and D, late-distant recurrence (>5 years) in patients recurrence-free at 5 years. HRs compare intermediate-risk (top) and high-risk (bottom) versus low-risk groups, respectively.

reach statistical significance, possibly due to a lower number of events in this cohort (Fig. 1A and B). Analysis of both cohorts combined (0–5 years, $n = 675$; 5–10 years, $n = 597$) showed a similar finding as in the multi-institutional cohort (Supplementary Fig. S5). These findings suggest that there are fundamentally two BCI risk groups for assessment of early and late recurrence: low/intermediate versus high for early recurrence risk and low versus intermediate/high for late recurrence risk. Because the BCI algorithm is linear and additive for H/I + MGI, an intermediate-risk profile can be attained by a range of tumor expression levels of H/I and MGI. Further analysis of the intermediate-risk group may contribute to a greater understanding of the profile for tumors that are indolent at diagnosis but have a greater propensity for late recurrence. Additional retrospective studies from randomized prospective clinical trials with a greater number of distant recurrence events are needed to clarify the performance of the intermediate-risk group.

The additive prognostic performance of BCI beyond standard clinicopathologic factors was also examined. Multivariate modeling indicates that BCI outperforms standard clinicopathologic factors for 0 to 5 years and more than 5-year risk in both the Stockholm TAM and multi-institutional cohorts. However, in the multi-institutional cohort, tumor size remained a significant prognostic factor for recurrence risk. This may be due to a comparatively greater proportion of larger tumors within the multi-institutional cohort versus the Stockholm TAM cohort. This finding is consistent with a reported retrospective study of 790 LN^- patients in which 15-year lethality rates increased with increasing tumor size (16).

Current standard of care diagnostic modalities used for assessment of recurrence risk contain algorithms that

Table 3. Early-distant recurrence: univariate and multivariate Cox model analysis after stepwise variable selection by AIC of likelihood of early-distant recurrence in Stockholm TAM and multi-institutional cohorts for all (ER^+) and $ER^+/HER2^-$ only patients

	All patients (ER^+)		$ER^+/HER2^-$ only	
Variable[a]	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Stockholm TAM	$n = 317$ (13 DR)		$n = 295$ (11 DR)	
Univariate analysis				
Age at surgery	0.76 (0.26–2.20)	0.62	0.59 (0.19–1.86)	0.37
Tumor size	1.68 (0.46–6.21)	0.44	2.44 (0.63–9.45)	0.20
Tumor grade	1.28 (0.28–5.84)	0.75	1.08 (0.23–5.08)	0.92
PR status	1.03 (0.28–3.79)	0.97	0.69 (0.18–2.66)	0.59
HER2 status	2.56 (0.56–11.68)	0.23	NA	NA
BCI	9.08 (1.86–44.4)	0.006	16.25 (2.59–102.0)	0.003
Multivariate analysis				
BCI	9.08 (1.86–44.4)	0.006	16.25 (2.59–102.0)	0.003
Multi-institutional	$n = 358$ (34 DR)		$n = 316$ (26 DR)	
Univariate analysis				
Age at surgery	1.10 (0.80–1.50)	0.57	1.16 (0.81–1.65)	0.42
Tumor size	1.77 (0.90–3.47)	0.10	1.49 (0.68–3.24)	0.32
Tumor grade	3.21 (0.98–10.51)	0.05	2.55 (0.77–8.49)	0.13
HER2 status	2.44 (1.10–5.39)	0.03	NA	NA
Chemo	0.99 (0.48–2.04)	0.98	1.08 (0.49–2.48)	0.86
BCI	11.92 (4.53–31.33)	<0.0001	13.71 (4.54–41.36)	<0.0001
Multivariate analysis				
Tumor grade	2.32 (0.71–7.58)	0.17	NA	NA
BCI	11.50 (4.22–31.32)	<0.0001	13.71 (4.54–41.36)	<0.0001

Abbreviations: AIC, Akaike's Information Criterion; DR, distant recurrences; NA, not applicable.

[a]Age was by 10 years increment; tumor size was greater than 2 cm versus 2 cm or less; tumor grade was moderate/poorly differentiated versus well differentiated; PR and HER2 status was positive versus negative; chemo was no versus yes; BCI was continuous variable with 5 units increment (chosen to dichotomize BCI to facilitate the comparison of HRs with clinical covariates).

integrate clinicopathologic factors (e.g., Adjuvant! Online) or are gene-expression–based algorithms (e.g., 21-gene, 70-gene) that generate an overall 0- to 10-year risk of recurrence. Although these algorithms generate an overall 0- to 10-year risk of recurrence, their clinical use is mostly limited to 0 to 5 years (5, 7). The time-dependent prognostic performance for these gene expression–based assays (21-gene, 70-gene) is fundamentally linked to these signatures primarily assessing tumor grade/proliferation (17). For example, in a cohort of ER^+ LN^- systemically untreated breast cancer patients, a tumor grade gene expression signature showed prognostic performance equivalent to that of the 70-gene signature (18). Notably, both signatures were predictive for the development of distant metastases within the first 5 years with decreasing prognostic ability beyond 5 years. Therefore, assessment of distant recurrence risk throughout a 10-year continuum requires not only quantification of tumor grade/proliferation status but also identification tumor characteristics that are associated with clinical late recurrence (>5 years; ref. 19).

BCI is an algorithmic gene expression–based signature that linearly integrates the tumor expression of the MGI and the expression ratio of HOXB13/IL17BR (H/I; refs. 9, 13, 20). MGI is the additive expression values of 5 cell-cycle genes for quantification of tumor grade and has been shown to be a significant prognostic for ER^+ LN^- patients (12). H/I was initially developed and validated as a significant prognostic factor independent of tumor grade in ER^+ LN^- tumor samples from prospectively obtained tumor banks and a single arm study of tamoxifen-treated patients from a randomized trial (11, 15, 21). Subsequent analysis of H/I expression in tumors from both arms of the randomized Stockholm cohort (untreated vs. tamoxifen-treated) indicate that high expression of H/I is both prognostic (untreated arm) and predictive of benefit from tamoxifen therapy ($P = 0.003$ for the interaction between treatment and continuous H/I; Supplementary Fig. S7). Herein, we report the development and validation of an optimized BCI for prediction of early- and late-distant recurrence risk by using the untreated patient arm from the Stockholm cohort as a training set. Within the untreated arm, H/I and MGI are both significant prognostic factors. However, these biomarkers have complementary performance for distant recurrence risk: MGI for 0 to 5 years, whereas H/I for more than 5 years

Table 4. Late-distant recurrence: univariate and multivariate Cox model analysis after stepwise variable selection by AIC of likelihood of late-distant recurrence in Stockholm TAM and multi-institutional cohorts for all (ER^+) and $ER^+/HER2^-$ patients

	All patients (ER^+)		$ER^+/HER2^-$ Only	
Variable[a]	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Stockholm TAM	$n = 285$ (20 DR)		$n = 266$ (18 DR)	
Univariate analysis				
Age at surgery	0.59 (0.26–1.34)	0.21	0.54 (0.23–1.29)	0.17
Tumor size	1.52 (0.50–4.57)	0.46	1.43 (0.41–4.99)	0.57
Tumor grade	0.69 (0.27–1.70)	0.41	0.87 (0.32–2.35)	0.78
PR status	0.45 (0.18–1.11)	0.08	0.50 (0.18–1.34)	0.17
HER2 status	1.38 (0.32–5.98)	0.67	NA	NA
BCI	4.07 (1.26–13.10)	0.019	4.92 (1.36–17.78)	0.015
Multivariate analysis				
PR status	0.52 (0.21–1.32)	0.17	0.54 (0.20–1.46)	0.22
BCI	3.50 (1.09–11.21)	0.035	4.57 (1.28–16.37)	0.020
Multi-Institutional	$n = 312$ (23 DR)		$n = 281$ (22 DR)	
Univariate analysis				
Age at surgery	1.32 (0.90–1.95)	0.16	1.48 (1.00–2.20)	0.05
Tumor size	3.37 (1.46–7.79)	0.01	3.17 (1.35–7.41)	0.01
Tumor grade	2.37 (0.88–6.38)	0.09	2.91 (0.98–8.58)	0.05
HER2 status	0.40 (0.05–2.98)	0.37	NA	NA
Chemo	0.89 (0.38–2.11)	0.80	1.04 (0.42–2.54)	0.94
BCI	8.01 (2.61–24.56)	0.0003	11.03 (3.36–36.21)	<0.0001
Multivariate analysis				
Tumor size	2.66 (1.14–6.20)	0.02	2.53 (1.07–5.97)	0.03
HER2 status	0.17 (0.02–1.32)	0.09	NA	NA
BCI	9.24 (2.85–30.00)	0.0002	9.33 (2.83–30.76)	0.0002

Abbreviations: DR, distant recurrences; NA, not applicable.

[a]Age was by 10 years increment; tumor size was greater than 2 cm versus 2 cm or less; tumor grade was poorly differentiated versus moderate/well differentiated; PR and HER2 status was positive versus negative; chemo was no versus yes; BCI was continuous variable with 5 units increment (chosen to dichotomize BCI to facilitate the comparison of HRs with clinical covariates).

(Supplementary Fig. S2). This observation is consistent with previous reports indicating proliferative status assessment (such as MGI) has limited prognostic performance for more than 5 years (5–8); thus, the sustainable prognostic performance of BCI is most likely due to the integration of prognostic information from both biomarkers.

Patients with hormone receptor–positive breast cancer who have received adjuvant endocrine therapy for 5 years have a continuous yearly recurrence rate extending out to 15 years with more than half of the recurrences occurring beyond 5 years from diagnosis (3). The National Cancer Institute of Canada Clinical Trials Group MA.17 trial, a randomized, placebo-controlled trial, showed that extended endocrine therapy with letrozole improves disease-free survival (DFS), distant DFS (DDFS), and overall survival in disease-free postmenopausal patients with hormone receptor–positive breast cancer following 5 years of tamoxifen (22, 23). Meta-analyses indicate that the vast majority ($\geq$90%) of ER^+ LN^- tamoxifen-treated patients are disease-free at 5 years, and therefore a decision is required whether to provide extended adjuvant endocrine therapy. Individual patient risk of distant recurrence at 5 years is of clinical importance given the comorbidities associated with endocrine therapies (24) and the recent results from the IDEAL randomized trial of extended endocrine therapy showing high patient noncompliance due to toxicities (25). Results reported herein indicate that BCI low-risk patients assessed at diagnosis continue to have a low probability of distant recurrence after 5 years, and therefore may be recommended to forgo extended therapy, whereas patients with BCI high risk may benefit from extended therapy.

One limitation of this study is that the multi-institutional cohort was not part of a prospective clinical trial and contained a subset of patients treated with adjuvant chemotherapy, which we accounted for in multivariate analyses. Another limitation is that the BCI prognostic performance was assessed in cohorts containing patients who received tamoxifen as the adjuvant endocrine therapy. Although aromatase inhibitors are the most commonly used adjuvant therapy in the United States,

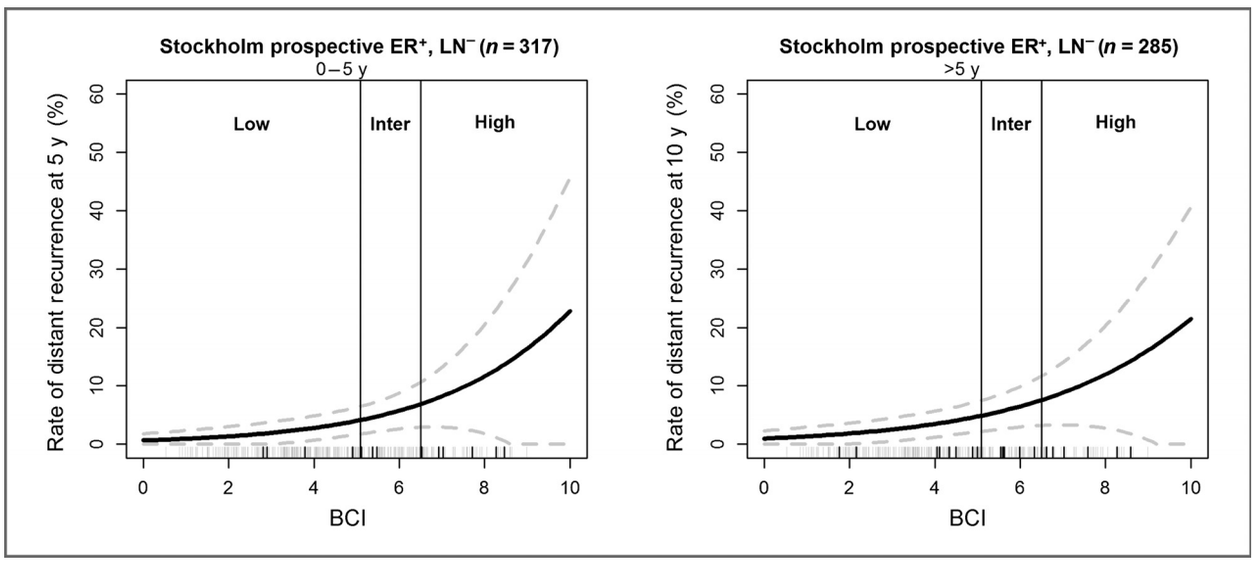

Figure 2. Continuous BCI score for early (0–5 years) and late (>5 years) rate of distant recurrence in the Stockholm TAM cohort (ER^+ LN^-). A, rate of early-distant recurrence at 5 years. B, rate of late-distant recurrence at 10 years in patients recurrence-free at 5 years. Gray and black rugged bars indicate individual patient BCI scores for nonrecurrence and recurrence respectively.

tamoxifen remains a common adjuvant therapy globally. Furthermore, recently reported results from a prospective randomized trial (Adjuvant Tamoxifen, Longer Against Shorter [ATLAS]) showed that continued tamoxifen therapy for 10 years versus stopping at 5 years further reduces recurrence rate and mortality, indicating the use of adjuvant tamoxifen therapy for 10 years from time of diagnosis (26).

BCI represents a next generation prognostic test with long-term sustainable clinical use for the prediction of distant recurrence for ER^+ LN^- patients that may allow for the avoidance of unnecessary adjuvant therapy and identify patients in most need of treatment. Management of patients with breast cancer requires individual prognostic information, which is fundamental for the decisions of chemotherapy, extended adjuvant endocrine therapy, and patient monitoring. The use of BCI at diagnosis should enable clinicians to more clearly assess recurrence risk within a time continuum that is aligned with the known horizon of breast cancer management.

Disclosure of Potential Conflicts of Interest

Y. Zhang, C.A. Schnabel, and B.E. Schroeder are employed as Director, Biostatistics, VP, Scientific Affairs & Clinical Development, and Director, Medical & Scientific Affairs, respectively, in bioTheranostics, Inc. and have ownership interest in the same. D. Sgroi has ownership interest in a patent. M. Erlander is employed as a Chief Scientific Officer and has stock options at bioTheranostics, Inc. No potential conflicts of interest were disclosed by the other authors.

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