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Breast Cancer Index Is a Predictive Biomarker of Treatment Benefit and Outcome from Extended Tamoxifen Therapy: Final Analysis of the Trans-aTTom Study

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ABSTRACT

Purpose: The Breast Cancer Index (BCI) *HOXB13/IL17BR* (H/I) ratio predicts benefit from extended endocrine therapy in hormone receptor–positive (HR^+) early-stage breast cancer. Here, we report the final analysis of the Trans-aTTom study examining BCI (H/I)'s predictive performance.

Experimental Design: BCI results were available for 2,445 aTTom trial patients. The primary endpoint of recurrence-free interval (RFI) and secondary endpoints of disease-free interval (DFI) and disease-free survival (DFS) were examined using Cox proportional hazards regression and log-rank test.

Results: Final analysis of the overall study population ($N = 2{,}445$) did not show a significant improvement in RFI with extended tamoxifen [HR, 0.90; 95% confidence interval (CI), 0.69–1.16; $P = 0.401$]. Both the overall study population and N0 group were underpowered due to the low event rate in the N0 group. In a pre-planned analysis of the N^+ subset ($N = 789$), BCI (H/I)-High patients derived significant benefit from extended tamoxifen (9.7% absolute benefit: HR, 0.33; 95% CI, 0.14–0.75; $P = 0.016$), whereas BCI (H/I)-Low patients did not (−1.2% absolute benefit; HR, 1.11; 95% CI, 0.76–1.64; $P = 0.581$). A significant treatment-to-biomarker interaction was demonstrated on the basis of RFI, DFI, and DFS ($P = 0.037$, 0.040, and 0.025, respectively). BCI (H/I)-High patients remained predictive of benefit from extended tamoxifen in the $N^+/HER2^-$ subgroup (9.4% absolute benefit: HR, 0.35; 95% CI, 0.15–0.81; $P = 0.047$). A three-way interaction evaluating BCI (H/I), treatment, and HER2 status was not statistically significant ($P = 0.849$).

Conclusions: Novel findings demonstrate that BCI (H/I) significantly predicts benefit from extended tamoxifen in HR^+ N^+ patients with $HER2^-$ disease. Moreover, BCI (H/I) demonstrates significant treatment to biomarker interaction across survival outcomes.

Introduction

The aTTom (Adjuvant Tamoxifen—To Offer More?) trial is a pivotal prospective phase III study that established the benefit of an additional 5 years of tamoxifen in patients with early-stage hormone receptor–positive (HR^+) breast cancer following the standard 5 years of adjuvant tamoxifen therapy (1). The aTTom trial randomized 6,953 patients to receive either 5 or 10 years of tamoxifen and demonstrated improved outcomes from the additional 5 years of tamoxifen with respect to disease-free interval (DFI) at a median 8.9 years of follow-up [HR, 0.86; 95% confidence interval (CI), 0.77–0.96; $P = 0.006$]. In addition, results showed that the impact of extended tamoxifen increased in a time-dependent manner: a reduction in breast cancer-related deaths was observed with increased duration of tamoxifen treatment after year 5 (1). Results from the aTTom trial were consistent with findings from other extended endocrine therapy trials, which reported modest benefits in absolute risk reduction with notable side effects and toxicities (2–4). At the same time, benefit from extended endocrine therapy is sensitive to the type, duration, and sequence of therapies administered (3, 5–7). Studies of extended tamoxifen therapy following primary adjuvant therapy with tamoxifen reported significant improvements in disease-free survival (DFS) of about 3.8% (1, 2). Trials that examined extended aromatase inhibitor (AI) therapy following primary adjuvant therapy with tamoxifen also reported benefit, in DFS (8) or in either recurrence-free interval (RFI) or recurrence-free survival (RFS; refs. 9, 10). However, results from investigations of extended AI therapy following primary adjuvant therapy that included an AI were mixed, with reports of both improvement in DFS (8) and no improvement in DFS (5, 11). Current clinical practice guidelines recommend up to 10 years of an AI for postmenopausal women with moderate to high risk based on clinicopathologic features and prognostic biomarkers (12). Multi-gene classifiers that provide insight into endocrine sensitivity and benefit may provide an individualized approach to evaluating risk versus benefit and guide de-escalation or extension of endocrine treatment.

The Breast Cancer Index (BCI) is a gene expression–based assay that integrates two components: the Molecular Grade Index (MGI) and the two-gene ratio *HOXB13/IL17BR* (H/I). MGI evaluates important

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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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Translational Relevance

The translational-aTTom (Trans-aTTom) study is a prospective–retrospective study designed to validate the ability of BCI to predict benefit from extended tamoxifen therapy in early-stage HR^+ breast cancer. In this final analysis, BCI (H/I) status significantly predicted benefit from 5 to 10 years of extended tamoxifen treatment with similar results and significant treatment by biomarker interaction in both the overall N^+ and $N^+/HER2^-$ cohorts. These data further strengthen the clinical evidence for BCI (H/I) as a predictive biomarker of extended endocrine benefit.

tumor proliferation pathways, whereas H/I assesses estrogen signaling in breast cancer. The BCI assay reports both a prognostic and a predictive result. The BCI score combines MGI and H/I to provide an individualized prognostic risk assessment for overall (0–10 years) and late (5–10 years) distant recurrence (13–15). The predictive component of BCI, BCI (H/I), has been shown to predict endocrine benefit across various treatment regimens that include tamoxifen or AIs (13, 14, 16, 17). BCI (H/I) was validated for prediction of extended endocrine benefit in previously reported results from the Translational-aTTom (Trans-aTTom) study (16). An important component of the Trans-aTTom study was the definitive confirmation of pathological subtype based on centralized assessment of estrogen receptor (ER), progesterone receptor (PR) expression, and HER2 overexpression, which was not determined within the parent trial. In the current study, updated and final analyses of the Trans-aTTom study and the impact of HER2 status on BCI (H/I)-predictive activity were evaluated.

Materials and Methods

Study design and patients

Trans-aTTom is a multi-institutional, prospective-retrospective study with the objective of validating BCI (H/I) as a predictive biomarker of extended endocrine therapy benefit in patients treated in the aTTom trial (16). All patients with available archival tumor specimens were included. Exclusion criteria included absence of invasive tumor as evaluated by histopathology review, insufficient tumor on tissue microarray (TMA) for IHC analysis, and insufficient RNA for BCI analysis (**Fig. 1**). Centralized collection and sample processing, construction of TMAs, and tissue sectioning was carried out by the University of Edinburgh Cancer Research Center as described previously (16).

ER, PR, and HER2 determination

Centralized IHC analysis was performed in a CLIA-certified laboratory at the Massachusetts General Hospital blinded to clinical data and outcome. The majority of patients from the parent aTTom trial had an unconfirmed HR status; therefore, determination of ER and PR status by IHC was performed on all cases as previously reported (16). IHC staining of TMAs was performed following standard protocols and scored using the Allred scoring system and the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (18) for ER/PR using ≥1% of positive cells as the cutoff value. Centralized HER2 status was determined for all cases using IHC and scored on a scale of 0–3+ with scores of 0 or 1+ being negative and a score of 3+ being positive. Equivocal HER2 scores of 2+ were resolved by FISH testing following current ASCO/CAP guidelines (19).

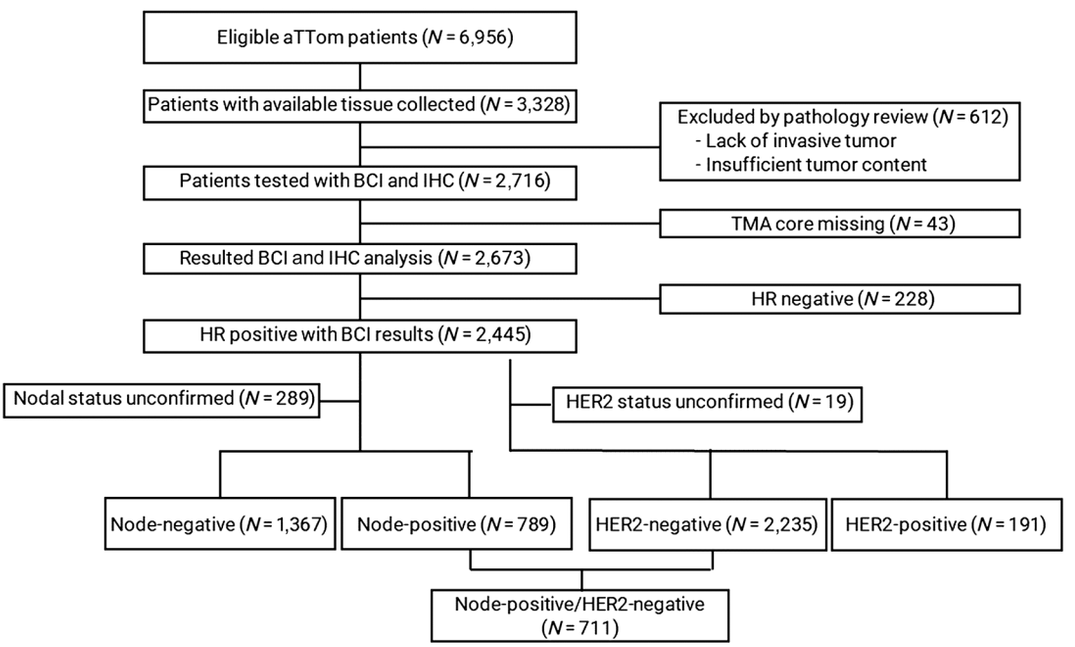

Figure 1.
Modified REMARK diagram. The diagram shows tumor block collection, specimen processing, and molecular testing, leading to a final updated analyzable cohort of 789 N^+ patients, including 711 who were HER2 negative.

Table 1. Clinicopathological characteristics for patients in the Trans-aTTom cohort.

	Trans-aTTom HR^+ ($n = 2,445$)	Trans-aTTom HR^+ N^+ ($n = 789$)
Age		
<50	237 (13%)	101 (13%)
50–59	719 (34%)	272 (34%)
60–69	795 (28%)	218 (28%)
≥70	694 (25%)	198 (25%)
Menopause		
Pre	63 (4%)	25 (3%)
Post	2,116 (86%)	679 (86%)
Peri	75 (3%)	28 (4%)
Not known	191 (7%)	57 (7%)
Nodal status		
Negative	1,367 (56%)	0
Positive	789 (32%)	789 (100%)
Unknown	289 (12%)	0
Tumor size		
T1	1,510 (46%)	362 (46%)
T2	711 (43%)	336 (43%)
T3	52 (4%)	30 (4%)
Not known	172 (8%)	61 (8%)
Histological grade		
Well differentiated	500 (15%)	118 (15%)
Moderately differentiated	1,036 (47%)	369 (47%)
Poorly differentiated	418 (20%)	161 (20%)
Not known	491 (18%)	141 (18%)
ER		
Negative	49 (2%)	17 (2%)
Positive	2,392 (98%)	771 (98%)
Not known	4 (0%)	1 (0%)
PR		
Negative	266 (9%)	69 (9%)
Positive	2,168 (91%)	717 (91%)
Not known	11 (0%)	3 (0%)
HER2		
Negative	2,235 (90%)	711 (90%)
Positive	191 (9%)	72 (9%)
Not known	19 (1%)	6 (1%)
Locoregional recurrence	199 (8%)	75 (10%)
Distant recurrence	358 (15%)	207 (26%)
New breast primary	94 (4%)	22 (3%)
Breast cancer death	309 (13%)	175 (22%)

BCI assay

BCI gene expression analysis by RT-PCR was performed on formalin-fixed paraffin-embedded (FFPE) primary tumor specimens (Biotheranostics Inc.) as reported previously (16). Briefly, macrodissection was performed on FFPE sections to enrich tumor content before RNA extraction. Total RNA was reverse transcribed, and the resulting cDNA was pre-amplified by PCR using the PreAmp Master Mix Kit (Thermo Fisher Scientific) before TaqMan PCR analysis. BCI (H/I) was calculated and Low and High BCI (H/I) categories were determined using the prespecified cutoff value point as described previously (16).

Study objectives and endpoints

The primary objective of the study was to assess BCI (H/I) status (High vs. Low) and prediction of extended endocrine benefit of 10 versus 5 years of tamoxifen treatment. The secondary objective was to determine the predictive performance of BCI (H/I) in the $HR^+/HER2^-$ subset. The primary endpoint was RFI, defined as the time from randomization to first local, regional, or distant recurrence. The secondary endpoints were DFI, defined as the time from randomization to first local, regional, distant recurrence, or new breast primary, and DFS, defined as time from randomization to first local, regional, distant recurrence, new breast primary, or breast cancer death.

Statistical considerations

The prospective power analysis has been described previously (16). Briefly, on the basis of the previously disclosed 4% absolute benefit of extending tamoxifen from 5 to 10 years at a median 8.9 years of follow-up (1), assuming 40% of patients classified as BCI (H/I)-High and 30% estimated attrition rate, approximately 2,500 patients would be required to obtain approximately 1,800 HR^+ evaluable patients to detect a 9.4% absolute benefit in the BCI (H/I)-High subset with 80% power.

To account for the deviation from proportional hazards due to the crossover in the Kaplan–Meier survival curves and delayed treatment effect of extended tamoxifen observed in the parent aTTom trial (16), Fleming-Harrington weighted log-rank test and Cox regression analysis using time varying coefficients were used to assess statistical significance of treatment effect within each of the BCI (H/I) categories. The absolute benefit was defined as the reduction in recurrence risk at 17 years (post randomization at year 5 with 12 years of follow-up). The likelihood ratio test was used to test for the statistical significance of extended tamoxifen treatment by biomarker interaction, as well as the three-way interaction among treatment, BCI (H/I) category, and HER2 status. All analyses were conducted on the basis of a prespecified statistical analysis plan (SAP) using Stata (version 15.1; https://www.stata.com) and R statistical package (version 3.5.2; http://www.r-project.org).

Prespecified rules for unblinding

Following the initial disclosure of the Trans-aTTom results reporting BCI (H/I) and significant prediction of extended endocrine benefit in N^+ patients (16), case collection continued in a pre-specified and blinded manner based on an estimated power of <50% observed for both the overall cohort and the node-negative (N0) subset. The current study reports the final analysis of the Trans-aTTom study and an updated analysis of the N^+ subset expanded to include 789 patients (16).

Data availability

The data analyzed in the current study are not publicly available because they contain patient data and proprietary information. Aggregated data analyzed in the study are included in the article. Qualified researchers may contact the corresponding author with reasonable requests to view additional data.

Results

Archival tumor specimens were collected from 3,328 patients across 62 aTTom clinical trial sites, representing 48% of the parent aTTom trial population (**Fig. 1**). A cohort of 2,445 patients had confirmed HR^+ status and BCI results, which included 1,367 N0 patients, 789 N^+ patients, and 289 patients with unconfirmed nodal status (**Fig. 1**). Kaplan–Meier analysis of both the overall cohort and N0 subset revealed a modest benefit of extended tamoxifen treatment (1.6% and 1.5% absolute benefit, $P = 0.571$ and 0.457, respectively; Supplementary Fig. S1A and S1B). Despite extensive tumor collection efforts that

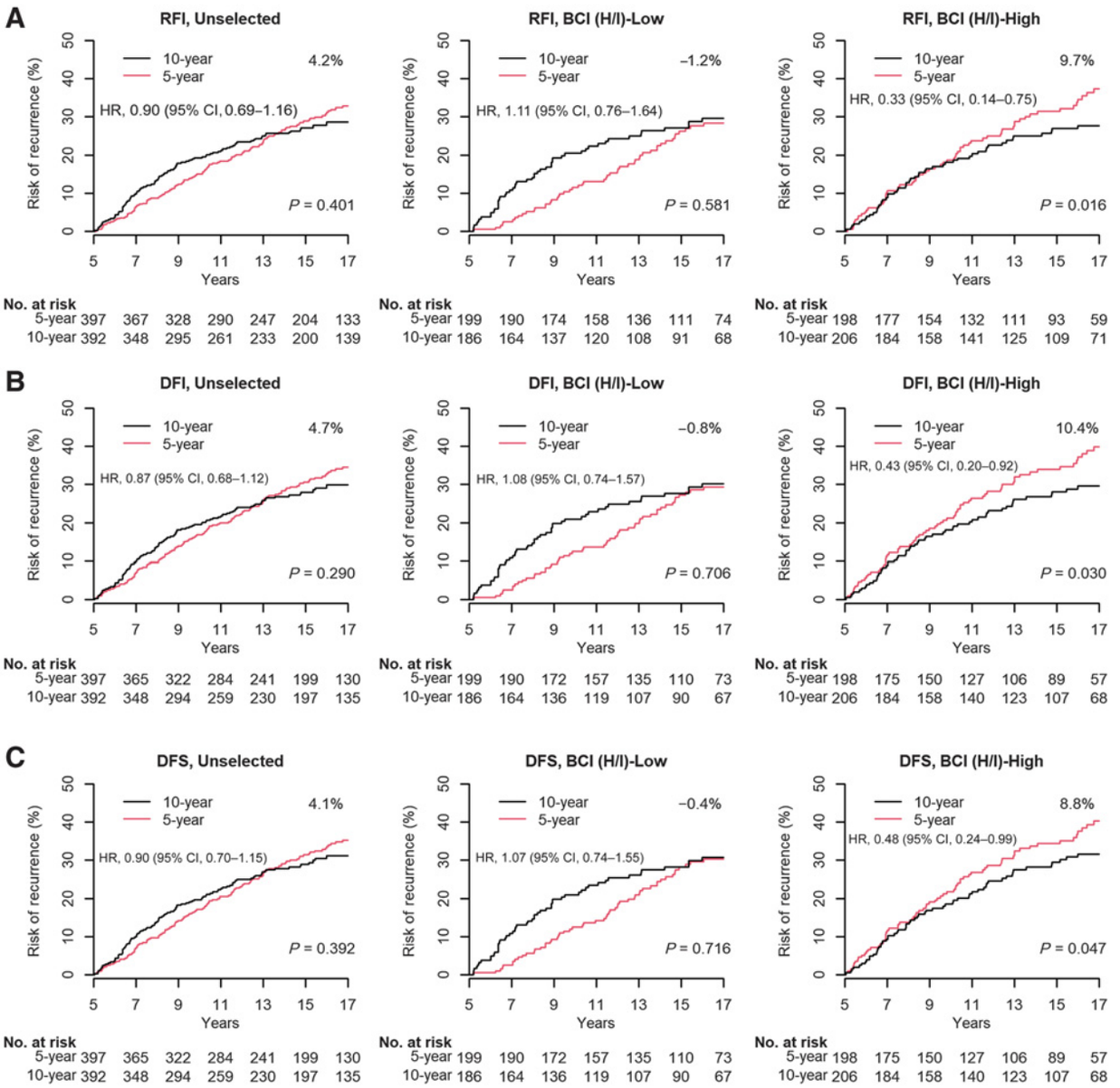

Figure 2.

Predictive performance by BCI (H/I) groups in N^+ subset ($n = 789$). Kaplan–Meier analysis of risk of recurrence comparing 10- with 5-year tamoxifen treatment based on RFI (**A**), DFI (**B**), and DFS (**C**).

were conducted over several years, the overall translational cohort and N0 subset remained underpowered (<30%) to evaluate BCI (H/I) due to the low event rate, 17-year recurrence rate of 13.2%, in the N0 subset. Results of analyses in this subset are presented in Supplementary Fig. S1B. However, given the lack of statistical power in this group no formal treatment by marker interaction tests were performed or reported. The analysis reported herein focused on the N^+ subset where statistical power was shown to be 94% to detect a treatment by biomarker interaction, extended adjuvant tamoxifen by BCI (H/I) status, and interaction at the $P = 0.05$ level for the primary endpoint (RFI). Additional analyses (DFI and DFS) were also performed on this subset.

BCI (H/I) is a predictive biomarker of extended endocrine benefit in N^+ patients

Final results included 789 N^+ patients, of which 86% were postmenopausal, 89% with T1 or T2 tumors, and 67% with moderately or poorly differentiated tumors. Ninty-eight percent were ER^+, 91% were PR^+, and 9% were $HER2^+$ (**Table 1**). Three hundred and ninty-seven patients received 5 years of tamoxifen with 125 recurrences in this group, whereas 392 patients received 10 years of tamoxifen with 106 recurrences. No significant improvement in RFI was observed in the N^+ group with extended tamoxifen treatment (HR, 0.90; 95% CI, 0.69–1.16; absolute benefit 4.2%; $P = 0.401$) with a 17-year recurrence risk of 28.6% (95% CI, 23.7–33.3) and 32.8% (95% CI, 27.6–37.8) for

Table 2. Kaplan–Meier estimates of 17-year risk of recurrence for patients treated with 10-year versus 5-year tamoxifen in all N^+ patients and in N^+ $HER2^-$ subset.

		10-year tamoxifen		5-year tamoxifen			
	Groups	**No. patients (%)**	**17-y risk (%; 95% CI, %)**	**No. patients (%)**	**17-y risk (%; 95% CI, %)**	**HR (95% CI)**	$P_{Interaction}$
All N^+ patients ($n = 789$)							
RFI	Unselected	392 (100%)	28.6 (23.7–33.3)	397 (100%)	32.8 (27.6–37.8)	0.90 (0.69–1.16)	
	BCI (H/I)-High	206 (53%)	27.7 (20.9–34.0)	198 (50%)	37.4 (29.4–44.6)	0.33 (0.14–0.75)	0.037
	BCI (H/I)-Low	186 (47%)	29.6 (22.2–36.3)	199 (50%)	28.4 (21.3–34.9)	1.11 (0.76–1.64)	
DFI	Unselected	392 (100%)	29.9 (24.9–34.6)	397 (100%)	34.6 (29.3–39.6)	0.87 (0.68–1.12)	
	BCI (H/I)-High	206 (53%)	29.6 (22.6–36.0)	198 (50%)	40.0 (31.8–47.1)	0.43 (0.20–0.92)	0.040
	BCI (H/I)-Low	186 (47%)	30.2 (22.7–36.9)	199 (50%)	29.4 (22.3–35.9)	1.08 (0.74–1.57)	
DFS	Unselected	392 (100%)	31.2 (26.1–36.0)	397 (100%)	35.3 (30.0–40.2)	0.90 (0.70–1.15)	
	BCI (H/I)-High	206 (53%)	31.6 (24.5–38.1)	198 (50%)	40.4 (32.2–47.5)	0.48 (0.24–0.99)	0.025
	BCI (H/I)-Low	186 (47%)	30.7 (23.2–37.5)	199 (50%)	30.3 (23.1–36.9)	1.07 (0.74–1.55)	
N^+ $HER2^-$ patients ($n = 711$)							
RFI	Unselected	359 (100%)	29.1 (23.9–34.0)	352 (100%)	32.4 (26.8–37.5)	0.92 (0.70–1.21)	
	BCI (H/I)-High	181 (50%)	27.7 (20.3–34.3)	161 (46%)	37.1 (28.2–44.8)	0.35 (0.15–0.81)	0.044
	BCI (H/I)-Low	178 (50%)	30.5 (22.8–37.5)	191 (54%)	28.4 (21.1–34.9)	1.15 (0.78–1.69)	
DFI	Unselected	359 (100%)	30.5 (25.2–35.5)	352 (100%)	34.3 (28.7–39.5)	0.88 (0.68–1.15)	
	BCI (H/I)-High	181 (50%)	29.8 (22.2–36.7)	161 (46%)	40.1 (31.1–47.9)	0.41 (0.18–0.91)	0.040
	BCI (H/I)-Low	178 (50%)	31.1 (23.4–38.1)	191 (54%)	29.4 (22.1–36.0)	1.10 (0.75–1.62)	
DFS	Unselected	359 (100%)	32.0 (26.6–37.0)	352 (100%)	35.1 (29.4–40.3)	0.91 (0.71–1.18)	
	BCI (H/I)-High	181 (50%)	32.1 (24.4–39.1)	161 (46%)	40.6 (31.6–48.4)	0.46 (0.22–0.98)	0.024
	BCI (H/I)-Low	178 (50%)	31.7 (23.9–38.7)	191 (54%)	30.4 (23.0–37.0)	1.10 (0.76–1.60)	

the 10- and 5-year arms, respectively (**Fig. 2A**; **Table 2**). The treatment effect of 10-year tamoxifen in the translational N^+ subset was similar to the effect reported in the N^+ subset of the aTTom parent cohort for the parent trial endpoint DFI (HR, 0.87; 95% CI, 0.68–1.12 for translational cohort vs. HR, 0.89; 95% CI, 0.76–1.05 for the parent cohort; refs. 1, 20).

For the primary endpoint of RFI, patients classified as BCI (H/I)-High (51%, $N = 404$) experienced significant benefit from 10 to 5 years of tamoxifen (HR, 0.33; 95% CI, 0.14–0.75). In contrast, there was no significant benefit from 10 to 5 years of tamoxifen in the 49% of patients ($N = 385$) classified as BCI-Low (HR, 1.11; 95% CI, 0.76–1.64; **Fig. 2A**). Furthermore, results evaluating BCI (H/I) as a continuous variable showed a significant treatment by biomarker interaction for the primary endpoint RFI ($P = 0.037$), adjusting for age, tumor size, grade, and PR status.

For secondary endpoints, BCI (H/I)-High N^+ patients who received extended tamoxifen treatment demonstrated a significant risk reduction for DFI (10.4% absolute benefit; HR, 0.43; 95% CI, 0.20–0.92; $P = 0.030$), whereas BCI (H/I)-Low patients did not (−0.8% absolute benefit; HR, 1.08; 95% CI, 0.74–1.57; $P = 0.706$; **Fig. 2B**; **Table 2**). Importantly, BCI (H/I)-High patients who received extended tamoxifen treatment further demonstrated a significantly improved outcome based on DFS (8.8% absolute benefit; HR, 0.48; 95% CI, 0.24–0.99; $P = 0.047$), whereas BCI (H/I)-Low patients did not (−0.4% absolute benefit; HR, 1.07; 95% CI, 0.74–1.55; $P = 0.716$; **Fig. 2C**; **Table 2**). Treatment by BCI (H/I) interaction was significant for both DFI ($P = 0.040$) and DFS ($P = 0.025$) endpoints.

The magnitude of endocrine benefit from extended tamoxifen observed in patients increased with rising levels of BCI (H/I) in the N^+ cohort (**Fig. 3A** and **B**). The risk of recurrence among patients with BCI (H/I)-High was 27.7% and 37.4% for patients treated with 10 and 5 years of tamoxifen, respectively, demonstrating a significant absolute benefit of 9.7% for reduction in the risk of recurrence ($P = 0.016$; **Fig. 2A**; **Table 2**). For patients with BCI (H/I)-Low, the risk of recurrence was 29.6% and 28.4% for patients treated with 10 and 5 years of tamoxifen, respectively, demonstrating a non-significant absolute risk reduction of −1.2% ($P = 0.581$; **Fig. 2A**; **Table 2**). No significant interaction was observed between treatment and the percentage of either ER ($P = 0.769$) or PR ($P = 0.703$) positively stained cells (**Fig. 3C–F**).

Centralized assessment of HER2 receptor status using ASCO/CAP guidelines identified 9% ($N = 72$) of tumors as $HER2^+$ in the N^+ subset. Analysis of the $HER2^-$ subset demonstrated similar results when compared with the overall N^+ cohort, showing that 48% of tumors were classified as BCI (H/I)-High and showed significant benefit from 10 to 5 years of tamoxifen for RFI (9.4% absolute benefit; HR, 0.35; 95% CI, 0.15-0.81; $P = 0.047$; **Fig. 4A**; **Table 2**), DFI (10.3% absolute benefit; HR, 0.41; 95% CI, 0.18-0.91; $P = 0.047$; **Fig. 4B**; **Table 2**), and DFS (8.5% absolute benefit; HR, 0.46; 95% CI, 0.22–0.98; $P = 0.045$; **Fig. 4C**; **Table 2**). BCI (H/I)-Low patients (52%) did not show benefit for RFI (−2.2% absolute benefit; HR, 1.15; 95% CI, 0.78–1.69; $P = 0.491$; **Fig. 4A**; **Table 2**), DFI (−1.7% absolute benefit; HR, 1.10; 95% CI, 0.75–1.62; $P = 0.612$; **Fig. 4B**; **Table 2**), or DFS (−1.3% absolute benefit; HR, 1.10; 95% CI, 0.76–1.60; $P = 0.623$; **Fig. 4C**; **Table 2**). Consistent with the overall N^+ population, treatment by BCI (H/I) interaction in N^+ $HER2^-$ subset remained significant for all three endpoints (RFI: $P = 0.044$; DFI: $P = 0.040$; DFS: $P = 0.024$), adjusting for age, tumor size, grade, and PR status.

Three-way interaction, including BCI (H/I) as a continuous variable, treatment duration, and HER2 status, did not demonstrate statistical significance ($P = 0.849$), indicating that the predictive ability of BCI is not dependent on HER2 status.

Discussion

Consistent with previously reported findings (16), this expanded analysis of Trans-aTTom patients confirmed, with increased precision, that BCI (H/I) status (High vs. Low) significantly predicted benefit from 5 to 10 years of tamoxifen treatment. BCI (H/I) identified

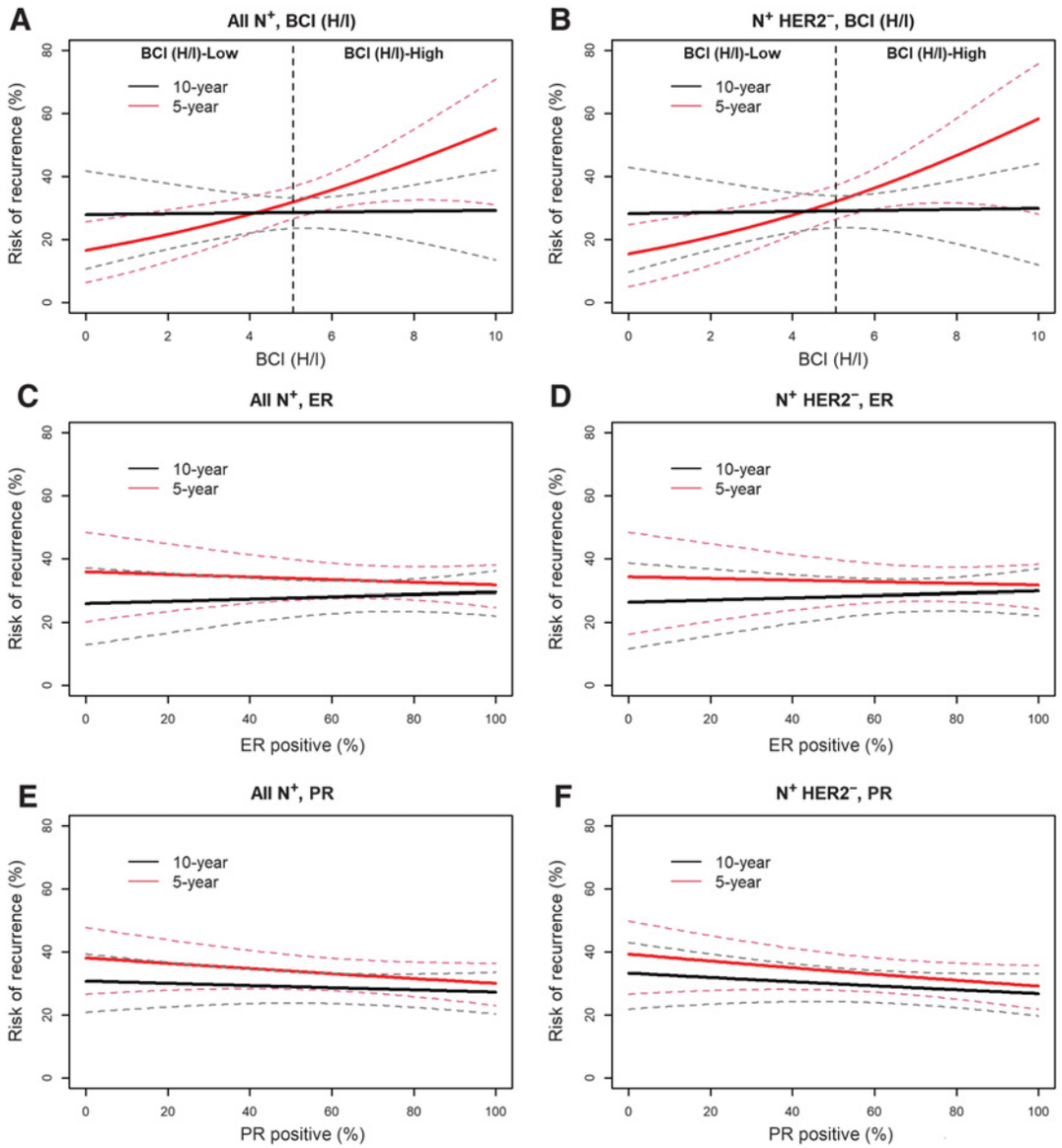

Figure 3.

Risk of recurrence as a function of continuous BCI (H/I), percent of ER-positive cells, and percent of PR-positive cells in all N^+ patients ($n = 789$) and in the N^+ $HER2^-$ subset ($n = 711$). Continuous risk curves as a function of BCI (H/I) for all N^+ patients (**A**) and N^+ $HER2^-$ subset (**B**). Continuous risk curves as a function of the percent of ER-positive cells for all N^+ patients (**C**) and N^+ $HER2^-$ subset (**D**). Continuous risk curves as a function of the percent of PR-positive cells for all N^+ patients (**E**) and N^+ $HER2^-$ subset (**F**).

approximately 50% of patients with N^+/HR^+ breast cancer that are unlikely to derive benefit from extended tamoxifen despite experiencing a higher risk of disease recurrence. Notably, BCI (H/I)-Low patients who received 10 years of tamoxifen therapy exhibited an increased risk of recurrence between years 5 and 15 (**Fig. 2**), suggesting that extended tamoxifen was potentially harmful in these patients (16). Furthermore, patients classified as BCI (H/I)-High showed a similar risk of recurrence between years 5 and 10, suggesting a carryover effect from the first 5 years of tamoxifen therapy. The carryover effect has been described previously by EBCTCG meta-analysis (4, 21) and was also observed in the recent NSABP B-42 BCI study (22). Results from the present study confirm that BCI (H/I) significantly stratifies tamoxifen benefit for the primary endpoint of RFI, as well as the additional endpoints of DFI and DFS, strengthening the evidence regarding treatment-to-biomarker interaction across a broader range of outcomes, including breast cancer–related death. These findings are clinically significant as they demonstrate that extended tamoxifen treatment in patients with BCI (H/I)-High disease leads to overall improved recurrence-free and disease-free outcomes, whereas patients with BCI (H/I)-Low disease may consider de-escalation to minimize exposure to toxicities and side effects associated with prolonged use of tamoxifen. Importantly, understanding the impact of extended endocrine therapy on survival endpoints may be critical to increasing patient compliance with extended medication and to ensure that

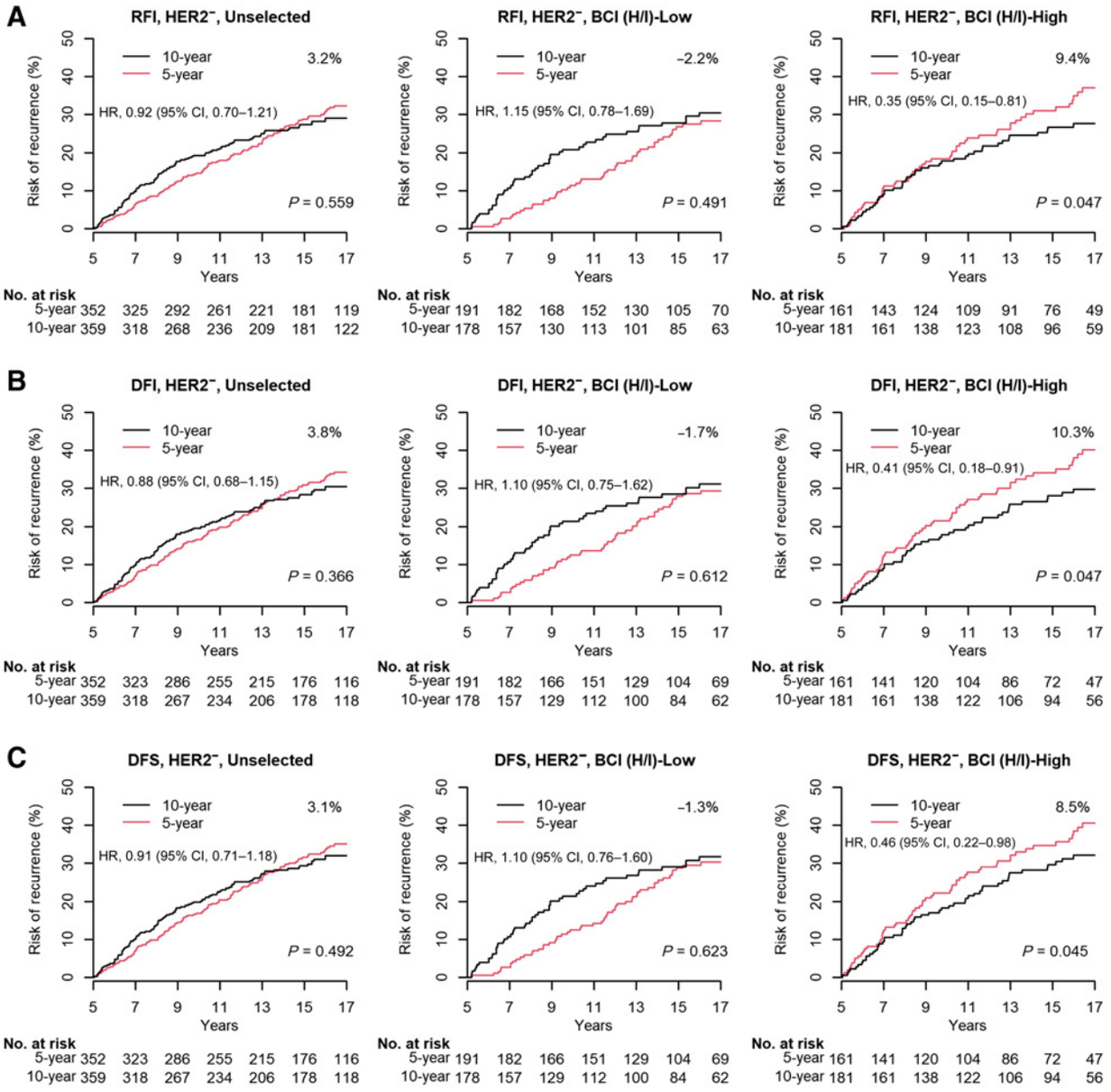

Figure 4.

Predictive performance by BCI (H/I) groups in N^+ $HER2^-$ subset ($n = 711$). Kaplan–Meier analysis of risk of recurrence comparing 10- with 5-year tamoxifen treatment based on RFI (**A**), DFI (**B**), and DFS (**C**).

patients that are endocrine responsive and at highest risk are carefully monitored and managed for tolerability and safety issues to improve adherence to treatment.

Although the Trans-aTTom study examined the predictive ability of BCI (H/I) in the context of extended tamoxifen therapy following primary adjuvant therapy with tamoxifen, BCI (H/I) has been demonstrated to predict endocrine benefit across several treatment regimens, including both tamoxifen and AIs (13, 16, 17). The ability of BCI (H/I) to predict extended endocrine therapy benefit has been shown for extended tamoxifen therapy following primary adjuvant tamoxifen in the Trans-aTTom trial (16), for extended AI therapy following primary adjuvant therapy with tamoxifen in the MA.17 trial (13), and for extended AI therapy following primary adjuvant therapy with an AI in the IDEAL trial (17). In the B-42 trial, which examined the sequence of an extended AI following primary adjuvant AI therapy, the predictive power of BCI (H/I) was less pronounced, but was significant following year 4 of extended AI therapy (22).

These results highlight the differences between prognostic and predictive biomarkers and underscore the clinical need for biomarkers predictive of response to endocrine therapy. Although other genomic classifiers, including Prosigna ROR, EPClin, and CTS5, have been extensively validated as prognostic biomarkers for late distant recurrence, predictive activity for response to extended endocrine therapy

has not been demonstrated (23, 24). More recently, in the NSABP B-42 trial, MammaPrint (MP) failed to meet the primary endpoint of distant recurrence in the predictive analysis and was not prognostic for late distant recurrence. The MP risk categories did appear to demonstrate prediction of extended letrozole therapy (ELT) benefit in the secondary endpoints of DFI and DFS but ELT benefit was associated with MP-Low instead of MP-High categorization and did not extend to distant recurrence prevention (25).

Additional findings from the current study examined BCI (H/I)-predictive activity in the context of HER2 disease status. Centralized HER2 assessment showed that 9% of Trans-aTTom patients were $HER2^+$ in the translational cohort, which is comparable with breast cancer epidemiological data (26). Approximately 50% of HER2-positive breast tumors are also ER/PR^+ (27, 28), and therefore would be treated with a combination of anti-estrogen and HER2-targeted therapies; knowledge of the degree of endocrine responsiveness in this subset may help refine treatment (29). Because of the known interactions between the ER and HER2 signaling pathways, one goal of this study was to determine whether HER2 status had any notable impact on BCI prediction of endocrine therapy benefit. Results presented herein indicate that BCI (H/I) showed similar predictive performance for extended endocrine benefit in the $N^+/HER2^-$ subset compared with the overall N^+ cohort, with a trend toward increased performance in the $HER2^-$ population. Although the $N^+/HER2^+$ subset was limited in size ($N = 72$), the three-way statistical interaction evaluating BCI (H/I), treatment, and HER2 status was not significant ($P = 0.849$), suggesting that signaling through the HER2 pathway does not extensively impact the ability of BCI (H/I) to predict benefit from extended tamoxifen. HER2 amplification has been shown to reduce sensitivity to anti-estrogen therapies by activating PI3K and MAPK pathways (29, 30). As molecular cross-talk between HER2 and ER contributes to the development of acquired resistance to hormonal therapy (31), the limited impact of HER2 status on BCI results in this setting is not unexpected, because BCI gene expression examines pre-treatment tumor biology. Additional studies are needed to further characterize BCI (H/I) biomarker effects in $HR^+/HER2^+$ disease treated with HER2-targeted therapies.

Limitations of this study include its retrospective nature, although the statistical analysis was prospectively defined, and all analyses were conducted blinded to clinical outcome. Despite substantial tumor tissue collection efforts of >3,000 patients representing 48% of the parent trial, the study remained underpowered to assess the BCI predictive effect in both the overall and node-negative patient cohorts due to low event rate and reduced treatment effect in the Trans-aTTom study population compared with the parent trial. Although previous BCI studies have included node-negative patients (17, 32, 33), additional studies are warranted to further characterize BCI predictive ability in node-negative disease, including meta-analyses across multiple studies. Although the $HER2^+$ percentage in this study was representative of the larger population, the small absolute size of the $HER2^+$ subset ($N = 191$) means that the impact of HER2 on BCI could only be measured indirectly by showing no difference in effect between the $HER2^+$ and $HER2^-$ subsets. Finally, this study consisted predominantly of post-menopausal women receiving tamoxifen monotherapy. Although tamoxifen remains a first-line treatment option for premenopausal patients and patients who cannot tolerate AI therapy, current guidelines in the United States recommend adjuvant endocrine therapy that includes an AI for postmenopausal patients (12). In this regard, BCI (H/I) status has been validated to predict benefit from extended AI treatment following primary adjuvant therapy with tamoxifen as demonstrated in MA.17 (13) or an AI as demonstrated in patients treated in the IDEAL trial (17).

In summary, BCI was predictive of endocrine response in this final updated Trans-aTTom analysis and identified a subset of HR^+/N^+ patients that experienced significant benefit, including increased DFS, from 10 to 5 years of tamoxifen therapy. These data, consistent with previous Trans-aTTom (16), MA.17 (13), and IDEAL reports (17), expand on the findings for BCI as a predictive biomarker of benefit from extended endocrine therapy. Together, these studies highlight BCI's unique ability to interrogate the underlying biology of endocrine responsiveness and provide additive molecular information independent of clinicopathologic factors that are traditionally used to guide treatment. On the basis of the collective evidence, the National Comprehensive Cancer Network (NCCN) breast cancer clinical practice guidelines recently recognized BCI (H/I) as a gene expression assay for prediction of benefit from extended endocrine therapy for both node-negative and node-positive patients across anti-estrogen therapies (34). Overall, findings from the present study further demonstrate the importance of identifying patients who are likely or unlikely to benefit from extended endocrine therapy and devising a treatment strategy based on genomic classification of individual endocrine response to improve quality of life and outcomes.

Authors' Disclosures

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