Breast Cancer Index is a predictive biomarker of treatment benefit and outcome from extended tamoxifen therapy: final analysis of the Trans-aTTom study

Breast Cancer Index is a predictive biomarker of treatment benefit and outcome from extended tamoxifen therapy: final analysis of the Trans-aTTom study

Running Title: BCI predicts endocrine benefit from extended tamoxifen

John M. S. Bartlett,[1,2] Dennis C. Sgroi,[3] Kai Treuner,[4] Yi Zhang,[4] Tammy Piper,[1] Ranelle C. Salunga,[4] Ikhlaaq Ahmed,[5] Lucy Doos,[5] Sarah Thornber,[5] Karen J. Taylor,[1] Elena F. Brachtel,[3] Sarah J. Pirrie,[5] Catherine A. Schnabel,[4] Daniel W. Rea[5]

[1]University Of Edinburgh, Edinburgh, United Kingdom, [2]Ontario Institute of Cancer Research, Ontario, [3]Massachusetts General Hospital, Boston, MA, USA, [4]Biotheranostics, Inc., San Diego, CA, USA, [5]University Of Birmingham, Cancer Research UK Clinical Trials Unit, Birmingham, United Kingdom.

Corresponding Author:

John M. S. Bartlett, PhD, FRCPath
Honorary Professor, The University of Edinburgh
Cancer Research UK Edinburgh Centre
Institute of Genetics and Cancer
The University of Edinburgh
Western General Hospital
Crewe Road South
Edinburgh EH4 2XR
T: 0131 651 8605
John.Bartlett@ed.ac.uk

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

JMSB receives honoraria from NanoString Technologies, Inc., Oncology Education, Biotheranostics, Inc., and MedcomXchange, Communications Inc. He is a consultant/advisor for Insight Genetics, Inc., BioNTech AG, Biotheranostics, Inc., Pfizer, Rna Diagnostics Inc., oncoXchange/MedcomXchange Communications Inc., Herbert Smith French Solicitors, and OncoCyte Corporation and receives travel support from Biotheranostics, Inc, NanoString Technologies, Inc., and Breast Cancer Society of Canada. He receives research funding from Thermo Fisher Scientific, Genoptix, Agendia, NanoString Technologies, Inc., Stratifyer GmbH, Biotheranostics, Inc. He holds the following patents: Dec 2020: Methods and Devices for Predicting Anthracycline Treatment Efficacy, EPO – 3169815 (granted); May 2021: Histone gene module predicts anthracycline benefit, USPTO – 11015226 (May 2021), EPO – 3359508 (Sept 2020) (granted); Jan 2017: Methods and Devices for Predicting Anthracycline Treatment Efficacy, US utility – 15/325,472; Canada – not yet assigned (applied for); Jan 2017: Systems, Devices and Methods for Constructing and Using a Biomarker, US utility – 15/328,108; EPO – 15824751.0; Canada – not yet assigned (applied for); Oct 2016: Histone gene module predicts anthracycline benefit, PCT/CA2016/000247 (applied for); Dec 2016: 95-Gene Signature of Residual Risk Following Endocrine Treatment, PCT/CA2016/000304 (applied for); Dec 2016: Immune Gene Signature Predicts Anthracycline Benefit, PCT/CA2016/000305 (applied for); June 2020: Use of Molecular Classifiers to Diagnose, Treat and Prognose Prostate Cancer, US Provisional 63/040.692 (applied for), Disclosure Name: A Molecular Classifier for Personalized Risk Stratification for Patients with Prostate Cancer, Date: 21/08/2019.

DCS, **YZ**, and **CAS** are named inventors on a patent to use *HOXB13/IL17BR* and Molecular Grade Index assays to predict breast cancer outcome.

KT, **YZ**, **RCS** and **CAS** are employees of Biotheranostics, Inc.

EFB receives research funding from Biotheranostics, Inc.

DWR receives honoraria from Genomic Health, Novartis, Pfizer, Roche, and research funding from Biotheranostics, Inc., Celgene, and Roche.

All remaining authors **TP**, **IA**, **LD**, **ST**, **KJT** and **SJP**, have declared no conflicts of interest.

STATEMENT OF 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 versus 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.

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 2445 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=2445) did not show a significant improvement in RFI with extended tamoxifen (HR=0.90; 95% 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 based on 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 early-stage HR+ breast cancer patients following the standard 5 years of adjuvant tamoxifen therapy (1). The aTTom trial randomized 6953 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% 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 deaths 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 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 RFI or RFS (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 aromatase inhibitor (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 tumor proliferation pathways, while 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 aromatase inhibitors (16–19). BCI (H/I) was validated for prediction of extended endocrine benefit in previously reported results from the Translational-aTTom (Trans-aTTom) study (18). 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, an 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

The translational aTTom study, 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 (18). 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 immunohistochemistry (IHC) analysis, and insufficient RNA for BCI analysis (**Figure 1**). Centralized collection and sample processing, construction of TMAs, and tissue sectioning was

carried out by the University of Edinburgh Cancer Research Center (ECRC) as described previously (18).

ER, PR and HER2 Determination

Centralized IHC analysis was performed in a CLIA-certified laboratory at the Massachusetts General Hospital (MGH) 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 (18). 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 (20) for ER/PR using ≥1% of positive cells as the cut-off. 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 (21).

BCI assay

BCI gene expression analysis by RT-PCR was performed on formalin-fixed paraffin-embedded (FFPE) primary tumor specimens (Biotheranostics Inc., San Diego, CA, USA) as reported previously (18). Briefly, macro-dissection 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, Carlsbad, CA, USA) prior to TaqMan PCR analysis. BCI (H/I) was calculated and Low and High BCI (H/I) categories were determined using the prespecified cut-point as described previously (18).

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 recurrence-free interval (RFI), defined as the time from randomization to first local, regional, or distant recurrence. The secondary endpoints were disease-free interval (DFI), defined as the time from randomization to first local, regional, distant recurrence or new breast primary and disease-free survival (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 (18). Briefly, based on the previously disclosed 4% absolute benefit of extending tamoxifen from 5 years 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 2500 patients would be required to obtain ~1800 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 (18), Fleming-Harrington weighted log-rank test and Cox regression analysis using time varying coefficients were utilized 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 based on a pre-specified 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 (18), 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 (18).

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 3328 patients across 62 aTTom clinical trial sites, representing 48% of the parent aTTom trial population (**Figure 1**). A cohort of 2445 patients had confirmed HR+ status and BCI results, which included 1367 N0 patients, 789 N+ patients, and 289 patients with unconfirmed nodal status (**Figure 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) (**Supplemental Figure 1A and B**). Despite extensive tumor collection efforts that 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 **Supplemental Figure 1B**. 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, extended adjuvant tamoxifen by BCI (H/I) status, 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 post-menopausal, 89% with T1 or T2 tumors, and 67% with moderately or poorly differentiated tumors. 98% were ER+, 91% were PR+, and 9% were HER2+ (**Table 1**). 397 patients received 5 years of tamoxifen with 125 recurrences in this group, while 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 the 10-year and 5-year arm, respectively (**Figure 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) (1,22).

For the primary endpoint of RFI, patients classified as BCI (H/I)-High (51%, N=404) experienced significant benefit from 10 vs 5 years of tamoxifen (HR=0.33; 95% CI, 0.14-0.75). In contrast, there was no significant benefit from 10 years vs 5 years of tamoxifen in the 49% of patients (N=385) classified as BCI-Low (HR=1.11; 95% CI, 0.76-1.64) (**Figure 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) (**Figure 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) (**Figure 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 (**Figure 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 years and 5 years of tamoxifen, respectively, demonstrating a significant absolute benefit of 9.7% for reduction in the risk of recurrence (*P*=0.016) (**Figure 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 years and 5 years

of tamoxifen, respectively, demonstrating a non-significant absolute risk reduction of -1.2% (*P*=0.581) (**Figure 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 (**Figure 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 to the overall N+ cohort, showing that 48% of tumors were classified as BCI (H/I)-High and showed significant benefit from 10 years versus 5 years of tamoxifen for RFI (9.4% absolute benefit; HR = 0.35; 95% CI, 0.15-0.81; *P*=0.047) (**Figure 4A**, **Table 2**), DFI (10.3% absolute benefit; HR=0.41; 95% CI, 0.18-0.91; *P*=0.047) (**Figure 4B**, **Table 2**), and DFS (8.5% absolute benefit; HR=0.46; 95% CI, 0.22-0.98; *P*=0.045) (**Figure 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) (**Figure 4A, Table 2**), DFI (-1.7% absolute benefit; HR=1.10; 95% CI, 0.75-1.62; *P*=0.612) (**Figure 4B**, **Table 2**) or DFS (-1.3% absolute benefit; HR=1.10; 95% CI, 0.76-1.60; *P*=0.623) (**Figure 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 (18), this expanded analysis of Trans-aTTom patients confirmed, with increased precision, that BCI (H/I) status (High vs Low) significantly predicted benefit from 5 vs 10 years of tamoxifen treatment. BCI (H/I) identified approximately 50% of N+/HR+ breast cancer patients 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 (**Figure 2**), suggesting that extended tamoxifen was potentially harmful in these patients (18). 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 (23,24) and was also observed in the recent NSABP B-42 BCI study (25). 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 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 patients that are endocrine responsive and at highest risk are carefully monitored and managed for tolerability and safety issues to improve adherence to treatment.

While 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 (16,18,19). 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 (18), 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 (19). 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 (13).

These results highlight the differences between prognostic and predictive biomarkers and underscore the clinical need for biomarkers predictive of response to endocrine therapy. While 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 (26,27). More recently, in the NSABP B-42 trial, MammaPrint (MP) failed to meet the primary endpoint of DR 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 (28).

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 (29). Approximately 50% of HER2-positive breast tumors are also ER/PR+ (30,31), 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

(32). Due to 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 to 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 phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (32,33). As molecular cross-talk between HER2 and ER contributes to the development of acquired resistance to hormonal therapy (34), the limited impact of HER2 status on BCI results in this setting is not unexpected, since 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 >3000 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 to the parent trial. While previous BCI studies have included node-negative patients (19,35,36), 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. While tamoxifen remains a first-line treatment option for pre-menopausal patients and patients who cannot tolerate AI therapy, current guidelines in the US 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 (19).

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 disease-free survival, from 10 versus 5 years of tamoxifen therapy. These data, consistent with previous Trans-aTTom (18) MA.17 (13), and IDEAL reports (19), 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 provides additive molecular information independent of clinicopathologic factors that are traditionally used to guide treatment. Based on 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 (37). 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.

ACKNOWLEDGMENTS

This work was supported by Biotheranostics, Inc., and in part by the Breast Cancer Research Foundation (to DCS grant numbers BCRF-17-145, BCRF-18-147, BCRF19-147, BCRF20-147) and the Ontario Institute for Cancer Research (grant number IA-036).

We extend sincere thanks to all the women who participated in the parent aTTom trial. We also thank the Principal Investigators and in particular the pathologists who contributed to this study: Dr Tarek Abdullah, Hairmyres Hospital; Dr Charlotte A Abson, Maidstone Hospital; Dr Douglas Adamson, Ninewells Hospital; Dr Fouad Alchami, University Hospital of Wales (Cardiff); Dr Hafiz Algurafi, Southend University Hospital; Dr Nisha Ali, Wythenshawe Hospital (Manchester); Dr David Bailey, Peterborough District Hospital; Miss Elizabeth Baker, Airedale General Hospital; Dr Catherine Bale, Ysbyty Gwynedd; Dr David Barker, Whiston Hospital (St Helen); Dr Urmila Barthakur, Yeovil District Hospital; Dr Gianfillipo Bertelli, Royal Sussex Hospital; Dr Jill Bishop, Glan Clwyd Hospital; Mr Riccardo Bonomi, Worthing Hospital; Dr Chris Bradley, Bradford Royal Infirmary; Dr Maurizio Brotto Singleton Hospital; Dr Jane Brown, Kent and Canterbury Hospital; Prof Adrian Murray Brunt, Royal Stoke University Hospital; Dr Mohammed Butt, Diana Princess of Wales Hospital; Dr David Butterworth, Macclesfield District General Hospital; Dr Pauline Carder, Bradford Royal Infirmary; Miss Leena S Chagla, Whiston Hospital (St Helen); Dr Alison Davies, Glan Clwyd Hospital; Dr Mark Davies, Singleton Hospital; Ms Eleri Davies, University Hospital of Wales (Cardiff) ; Dr Rahul Deb, Royal Derby Hospital; Dr Smita Deshpande, Manor Hospital (Walsall Hospital); Miss Sue Down, James Paget Hospital; Dr Sidharth Dubey, Derriford Hospital; Dr Janet English, Mid Yorkshire Hospital; Miss Abigail Alexandra Evans, Poole Hospital; Dr Indrajit N Fernando, Birmingham Heartlands Hospital; Dr David Fish, Maidstone Hospital; Dr Sunna Frank, Yeovil District Hospital; Dr Frances Gallagher, Hairmyres Hospital; Mr Chris Gateley, Royal Gwent Hospital; Dr Konstantinos Geropantas, Norfolk And Norwich University Hospital; Dr Andrew Goodman, Royal Devon and Exeter

Hospital; Dr Andrew Goodman, Torbay; Dr Preethi Gopinath, St Margaret's Hospital; Alison Green, Derriford Hospital; Dr Lisette Hammond, Royal Stoke University Hospital; Prof Andy Hanby, St James's University Hospital (Leeds); Mrs Claudia E Harding-Mackean, Countess of Chester Hospital; Miss Fiona Hoar, City Hospital (and Sandwell hospital); Mr Chris Holcombe, Royal Liverpool University Hospital; Lesley Hortan, Birmingham Heartlands Hospital; Dr Ehab Husain, Aberdeen Royal Infirmary; Mrs Anita Immanuel, Essex County Hospital (Colchester); Dr Mona Jain, Sunderland Royal Hospital; Dr Kamarul Jamil, St Mary's Hospital; Dr Jalal Kokan, Macclesfield District General Hospital; Dr Vidga Kuymaraswamy, Huddersfield Royal Infirmary; Dr Iain Macpherson, Glasgow Royal Infirmary; Dr Andreas Makris, Mount Vernon Hospital; Dr Jennifer Marshall, St Mary's Hospital; Mr Lee Martin, University Hospital Aintree; Dr Guy Martland, Poole Hospital; Dr Karen McAdam, Peterborough District Hospital; Dr Rakesh Mehra, New Cross Hospital (Wolverhampton); Dr Natalie Meara, Countess of Chester Hospital; Dr Yasmeen Mir, Royal Liverpool University Hospital; Dr Navid Momtahan, City Hospital; Dr Margaret Moody, West Suffolk Hospital; Dr Iqtedar Muazzam, Scunthorpe Hospital; Dr Narenda Mungalsingh, Wycombe General Hospital; Dr Joseph Murphy, Norfolk And Norwich University Hospital; Dr Claire Murray, Royal Devon and Exeter Hospital; Dr David Murray; Dr Shirin Namini, Mid Yorkshire Hospitals; Stephanie Needham, Royal Sussex Hospital; Dr Ashutosh Nerurkar, Royal Marsden Hospital Sutton; Dr Jonathan J Nicoll, West Cumberland Hospital; Dr John O'Dowd, Airedale General Hospital; Dr Gary Parves; Mr Ashraf Patel, St Margaret's Hospital; Prof Timothy J Perren, St James's University Hospital (Leeds); Dr Mojca Persic, Queen's Hospital (Burton); Demetris Poyiatzis, Bristol Haematology And Oncology Centre; Dr Elena Provenzano, Addenbrooke's Hospital; Dr Colin Purdie, Ninewells Hospital; Dr Sanjay Raj, Royal Hampshire County Hospital; Dr Lilani Ranasigne, West Suffolk Hospital; Dr Majid Rashid, Royal Gwent Hospital; Mr Zenon Rayter, Bristol Haematology And Oncology Centre; Prof Daniel W Rea, The Queen Elizabeth Hospital (Birmingham); Dr Sarah Read-Jones, Coventry and Warwickshire Hospital; Mrs Lisa Richardson, Manor Hospital (Walsall Hospital); Dr David

Rowlands; Dr Nick Ryley, Torbay; Ms Luise Seargent, Southend University Hospital; Dr Abeer Shaaban, Birmingham Heartlands Hospital; Dr Ketan Shah, Wycombe General Hospital; Dr Win Soe, Wrexham Maelor Hospital; Dr Balvinder Shoker, Royal Hampshire County Hospital; Dr Navita Somaiah, Royal Marsden Hospital Sutton; Dr Sophia Stanford, Basingstoke and North Hampshire Hospital; Dr Sandra D Tinkler, Basingstoke and North Hampshire Hospital; Dr Mark W Verrill, Newcastle General Hospital; Andrew Wagerfield, Essex County Hospital (Colchester); Professor Andrew Wardley, Wythenshawe Hospital (Manchester); Mr Malcolm West, Queen's Hospital (Burton); Dr Matthew Winter, Royal Hallamshire Hospital (Sheffield); Dr Cate Wight, Kent and Canterbury Hospital; Ms Kathryn Wright, Sunderland Royal Hospital.; Dr Fergus Young, West Cumberland Hospital

REFERENCES

1. Gray RG, Rea D, Handley K, Bowden SJ, Perry P, Earl HM, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol. 2013;31:5.

2. Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet. 2013;381:805–16.

3. Del Mastro L, Mansutti M, Bisagni G, Ponzone R, Durando A, Amaducci L, et al. Extended therapy with letrozole as adjuvant treatment of postmenopausal patients with early-stage breast cancer: a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 22:1458–67.

4. Bradley R, Burrett J, Clarke M, Davies C, Duane F, Evans V, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet. 2015;386:1341–52.

5. Tjan-Heijnen VCG, van Hellemond IEG, Peer PGM, Swinkels ACP, Smorenburg CH, van der Sangen MJC, et al. Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): a randomised, phase 3 trial. Lancet Oncol. 2017;18:1502–11.

6. De Placido S, Gallo C, De Laurentiis M, Bisagni G, Arpino G, Sarobba MG, et al. Adjuvant anastrozole versus exemestane versus letrozole, upfront or after 2 years of

tamoxifen, in endocrine-sensitive breast cancer (FATA-GIM3): a randomised, phase 3 trial. Lancet Oncol. 2018;19:474–85.

7. Jakesz R, Jonat W, Gnant M, Mittlboeck M, Greil R, Tausch C, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: Combined results of ABCSG trial 8 and ARNO 95 trial. Lancet. 2005;366:455–62.

8. Goss PE, Ingle JN, Pritchard KI, Robert NJ, Muss H, Gralow J, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med. 2016;375:209.

9. Mamounas EP, Jeong J-HH, Lawrence Wickerham D, Smith RE, Ganz PA, Land SR, et al. Benefit From Exemestane As Extended Adjuvant Therapy After 5 Years ofAdjuvant Tamoxifen: Intention-to-Treat Analysis of the National Surgical Adjuvant Breast and Bowel Project B-33 Trial. J Clin Oncol. 2008;26:1965–71.

10. Jakesz R, Greil R, Gnant M, Schmid M, Kwasny W, Kubista E, et al. Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: Results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. J Natl Cancer Inst. 2007;99:1845–53.

11. Gnant M, Fitzal F, Rinnerthaler G, Steger GG, Greil-Ressler S, Balic M, et al. Duration of Adjuvant Aromatase-Inhibitor Therapy in Postmenopausal Breast Cancer. N Engl J Med. 2021;385:395–405.

12. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KA, et al. Adjuvant endocrine therapy for women with hormone receptor–positive breast cancer:

ASCO clinical practice guideline focused update. J Clin Oncol. 2019;37:423–38.

13. Sgroi DC, Carney E, Zarrella E, Steffel L, Binns SN, Finkelstein DM, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst. 2013;105:1036–42.

14. Zhang Y, Schnabel CA, Schroeder BE, Jerevall P-LL, Jankowitz RC, Fornander T, et al. Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. Clin Cancer Res. 2013;19:4196–205.

15. Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol. 2013;14:1067–76.

16. Sgroi DC, Carney E, Zarrella E, Steffel L, Binns SN, Finkelstein DM, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst. 2013;105:1036–42.

17. Zhang Y, Schnabel CA, Schroeder BE, Jerevall PL, Jankowitz RC, Fornander T, et al. Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. Clin Cancer Res. 2013;19:4196–205.

18. Bartlett JMS, Sgroi DC, Treuner K, Zhang Y, Ahmed I, Piper T, et al. Breast Cancer Index and prediction of benefit from extended endocrine therapy in breast cancer patients treated in the Adjuvant Tamoxifen—To Offer More? (aTTom) trial. Ann Oncol. 2019;30:1776–83.

19. Noordhoek I, Treuner K, Putter H, Zhang Y, Wong J, Kranenbarg EMK, et al. Breast cancer index predicts extended endocrine benefit to individualize selection of patients with HR+ early-stage breast cancer for 10 years of endocrine therapy. Clin Cancer Res. 2021;27:311–9.

20. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol. 2020;38:1346–66.

21. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: American society of clinical oncology/college of American pathologists guideline update. Arch Pathol Lab Med. 2020;144:545–63.

22. Rea D, Gray R, Bowden S, Handley K, Earl H, Poole C, et al. Overall and subgroup findings of the aTTom trial: A randomised comparison of continuing adjuvant tamoxifen to 10 years compared to stopping after 5 years in 6953 women with ER positive or ER untested early breast cancer. Eur J Cancer. 2013;49:S402.

23. Abe O, Abe R, Enomoto K, Kikuchi K, Koyama H, Masuda H, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:60993–8.

24. Bradley R, Burrett J, Clarke M, Davies C, Duane F, Evans V, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet. 2015;386:1341–52.

25. Mamounas EP, Bandos H, Rastogi P, Zhang Y, Treuner K, Lucas PC, et al. Breast Cancer Index (BCI) and prediction of benefit from extended aromatase inhibitor (AI) therapy (tx) in HR+ breast cancer: NRG oncology/NSABP B-42. J Clin Oncol. 2021;39(15_suppl):501.

26. Bartlett JMS, Bayani J, Marshall A, Dunn JA, Campbell A, Cunningham C, et al. Comparing Breast Cancer Multiparameter Tests in the OPTIMA Prelim Trial: No Test Is More Equal Than the Others. J Natl Cancer Inst. 2016;108.

27. Sestak I, Buus R, Cuzick J, Dubsky P, Kronenwett R, Denkert C, et al. Comparison of the performance of 6 prognostic signatures for estrogen receptor–positive breast cancer a secondary analysis of a randomized clinical trial. JAMA Oncol. 2018;4:545–53.

28. Rastogi P, Bandos H, Lucas PC, Veer L van 't, Wei J-PJ, Geyer CE, et al. Utility of the 70-gene MammaPrint assay for prediction of benefit from extended letrozole therapy (ELT) in the NRG Oncology/NSABP B-42 trial. J Clin Oncol. 2021;39(15_suppl):502.

29. Female Breast Cancer Subtypes — Cancer Stat Facts. Available from: https://seer.cancer.gov/statfacts/html/breast-subtypes.html

30. Akashi M, Yamaguchi R, Kusano H, Obara H, Yamaguchi M, Toh U, et al. Diverse histomorphology of HER2-positive breast carcinomas based on differential ER expression. Histopathology. 2020;76:560–71.

31. Morita M, Yamaguchi R, Tanaka M, Tse GM, Yamaguchi M, Otsuka H, et al. Two progressive pathways of microinvasive carcinoma: Low-grade luminal pathway and high-grade HER2 pathway based on high tumour-infiltrating lymphocytes. J Clin Pathol.

2016;69:890–8.

32. Hanker AB, Sudhan DR, Arteaga CL. Overcoming Endocrine Resistance in Breast Cancer. Cancer Cell. 2020;37:496–513.

33. Kurokawa H, Lenferink AEG, Simpson JF, Pisacane PI, Sliwkowski MX, Forbes JT, et al. Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. Cancer Res. 2000;60:5887–94.

34. Haque MM, Desai K V. Pathways to Endocrine Therapy Resistance in Breast Cancer. Front Endocrinol (Lausanne). 2019;10:573.

35. Habel LA, Sakoda LC, Achacoso N, Ma XJ, Erlander MG, Sgroi DC, et al. HOXB13: IL17BR and molecular grade index and risk of breast cancer death among patients with lymph node-negative invasive disease. Breast Cancer Res. 2013;15:R24.

36. Schroeder B, Zhang Y, Stål O, Fornander T, Brufsky A, Sgroi DC, et al. Risk stratification with Breast Cancer Index for late distant recurrence in patients with clinically low-risk (T1N0) estrogen receptor-positive breast cancer. Nature. 2017;3:1–3.

37. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Breast Cancer v1.2021. 2021.

TABLES AND FIGURES

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

	Trans-aTTom HR+ (n=2445)	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	2116 (86%)	679 (86%)
Peri	75 (3%)	28 (4%)
Not known	191 (7%)	57 (7%)
Nodal Status		
Negative	1367 (56%)	0
Positive	789 (32%)	789 (100%)
Unknown	289 (12%)	0
Tumor Size		
T1	1510 (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	1036 (47%)	369 (47%)
Poorly differentiated	418 (20%)	161 (20%)
Not known	491 (18%)	141 (18%)
ER		
Negative	49 (2%)	17 (2%)
Positive	2392 (98%)	771 (98%)
Not known	4 (0%)	1 (0%)
PR		
Negative	266 (9%)	69 (9%)
Positive	2168 (91%)	717 (91%)
Not known	11 (0%)	3 (0%)
HER2		
Negative	2235 (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%)

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.

	Groups	10-Year Tamoxifen		5-Year Tamoxifen		HR (95% CI) *	Interaction P Value
		No. Patients (%)	17-yr Risk % (95% CI, %)	No. Patients (%)	17-yr Risk % (95% CI, %)		
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)	

Figure 1. Modified REMARK CONSORT 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.

Figure 2. Predictive performance by BCI (H/I) groups in N+ subset (n=789).
Kaplan-Meier analysis of risk of recurrence comparing 10-year versus 5-year tamoxifen treatment based on RFI (A), DFI (B), and DFS (C).

Figure 3. Risk of recurrence as a function of continuous BCI (H/I) 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 (D) and N+ HER2- subset (E).

Figure 4. Predictive performance by BCI (H/I) groups in N+ HER2- subset (n=711).
Kaplan-Meier analysis of risk of recurrence comparing 10-year versus 5-year tamoxifen treatment based on RFI (A), DFI (B), and DFS (C).

Figure 1

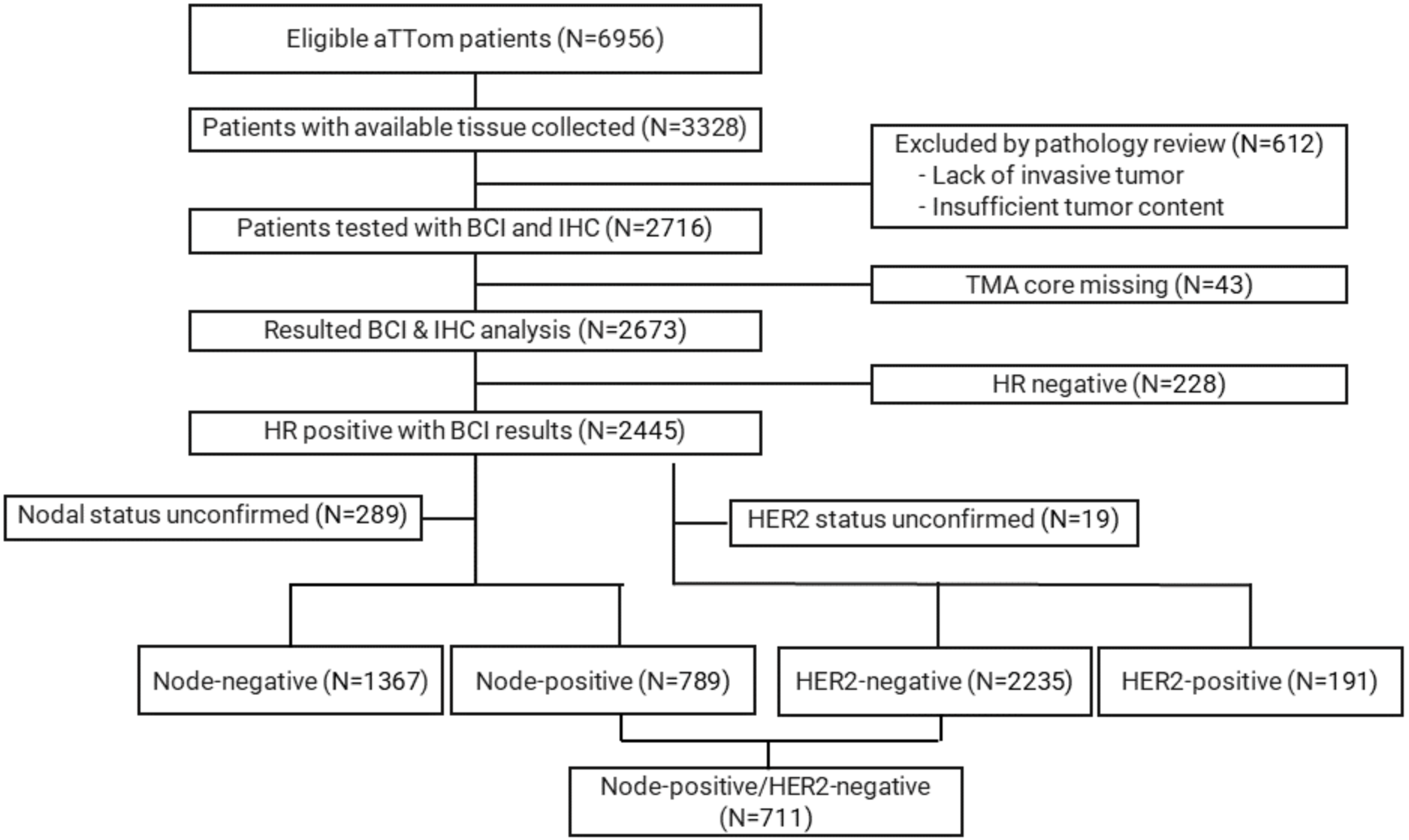

Eligible aTTom patients (N=6956)
Patients with available tissue collected (N=3328)
Excluded by pathology review (N=612)
- Lack of invasive tumor
- Insufficient tumor content
Patients tested with BCI and IHC (N=2716)
TMA core missing (N=43)
Resulted BCI & IHC analysis (N=2673)
HR negative (N=228)
HR positive with BCI results (N=2445)
Nodal status unconfirmed (N=289)
HER2 status unconfirmed (N=19)
Node-negative (N=1367)
Node-positive (N=789)
HER2-negative (N=2235)
HER2-positive (N=191)
Node-positive/HER2-negative (N=711)

Figure 2

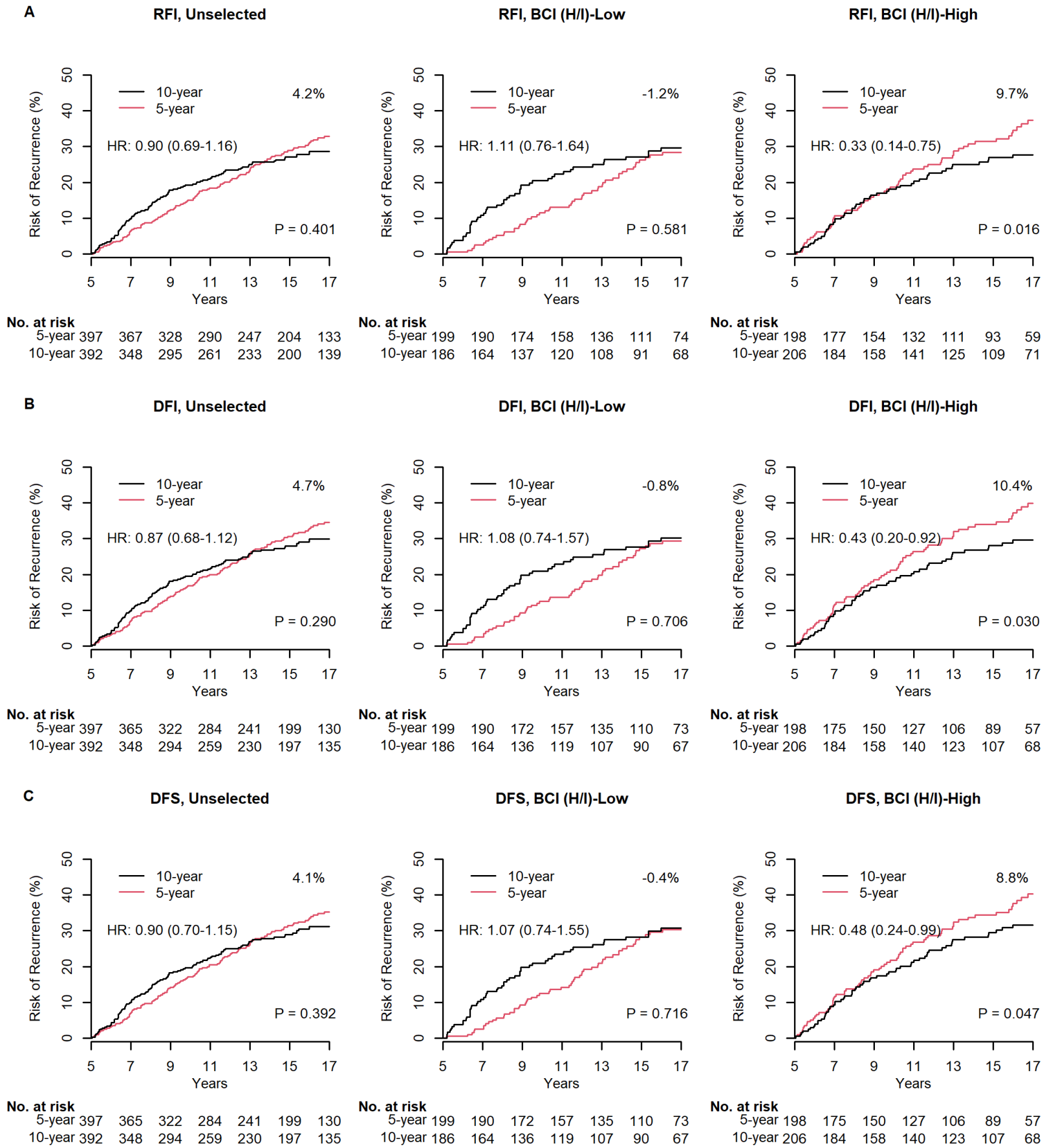

A
RFI, Unselected
RFI, BCI (H/I)-Low
RFI, BCI (H/I)-High
10-year
5-year
4.2%
-1.2%
9.7%
HR: 0.90 (0.69-1.16)
HR: 1.11 (0.76-1.64)
HR: 0.33 (0.14-0.75)
P = 0.401
P = 0.581
P = 0.016
Risk of Recurrence (%)
Years
No. at risk
5-year 397 367 328 290 247 204 133
10-year 392 348 295 261 233 200 139
5-year 199 190 174 158 136 111 74
10-year 186 164 137 120 108 91 68
5-year 198 177 154 132 111 93 59
10-year 206 184 158 141 125 109 71
B
DFI, Unselected
DFI, BCI (H/I)-Low
DFI, BCI (H/I)-High
4.7%
-0.8%
10.4%
HR: 0.87 (0.68-1.12)
HR: 1.08 (0.74-1.57)
HR: 0.43 (0.20-0.92)
P = 0.290
P = 0.706
P = 0.030
5-year 397 365 322 284 241 199 130
10-year 392 348 294 259 230 197 135
5-year 199 190 172 157 135 110 73
10-year 186 164 136 119 107 90 67
5-year 198 175 150 127 106 89 57
10-year 206 184 158 140 123 107 68
C
DFS, Unselected
DFS, BCI (H/I)-Low
DFS, BCI (H/I)-High
4.1%
-0.4%
8.8%
HR: 0.90 (0.70-1.15)
HR: 1.07 (0.74-1.55)
HR: 0.48 (0.24-0.99)
P = 0.392
P = 0.716
P = 0.047
5-year 397 365 322 284 241 199 130
10-year 392 348 294 259 230 197 135
5-year 199 190 172 157 135 110 73
10-year 186 164 136 119 107 90 67
5-year 198 175 150 127 106 89 57
10-year 206 184 158 140 123 107 68

Figure 3

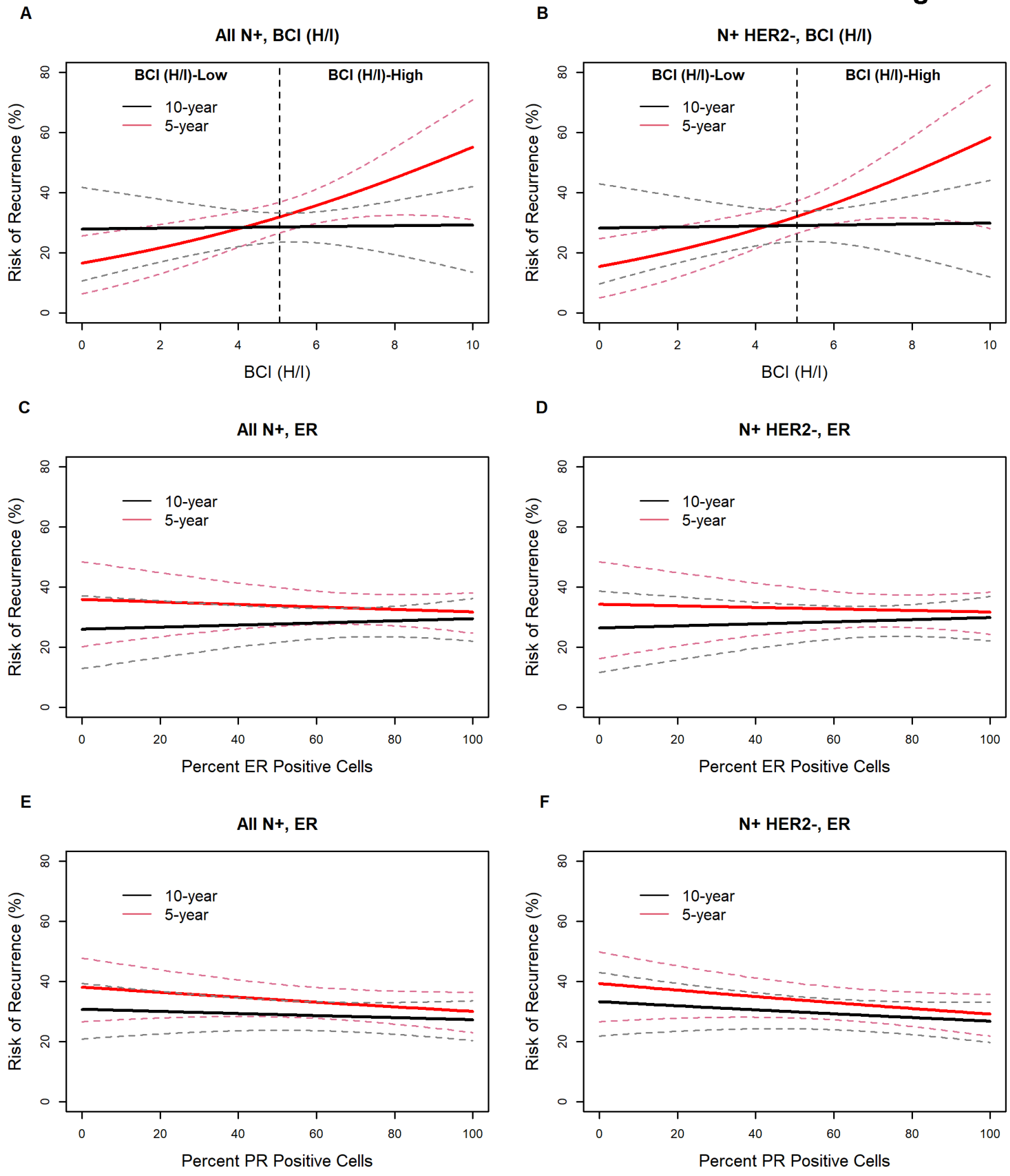
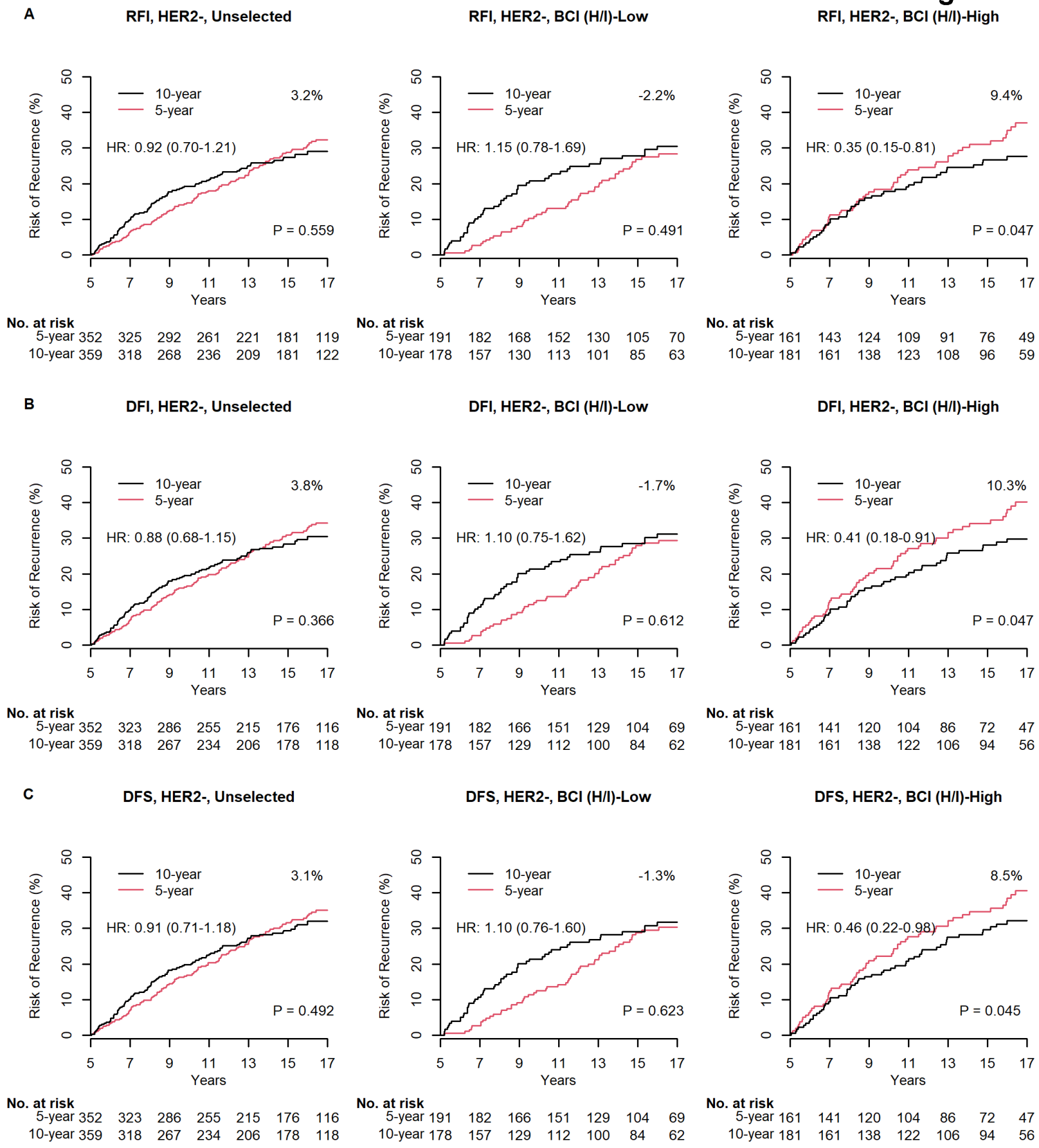

A
All N+, BCI (H/I)
BCI (H/I)-Low
BCI (H/I)-High
10-year
5-year
Risk of Recurrence (%)
BCI (H/I)
B
N+ HER2-, BCI (H/I)
C
All N+, ER
Percent ER Positive Cells
D
N+ HER2-, ER
E
All N+, ER
Percent PR Positive Cells
F
N+ HER2-, ER

Figure 4


A
RFI, HER2-, Unselected
10-year
5-year
3.2%
HR: 0.92 (0.70-1.21)
P = 0.559
Risk of Recurrence (%)
Years
No. at risk
5-year 352 325 292 261 221 181 119
10-year 359 318 268 236 209 181 122
RFI, HER2-, BCI (H/I)-Low
10-year
5-year
-2.2%
HR: 1.15 (0.78-1.69)
P = 0.491
Risk of Recurrence (%)
Years
No. at risk
5-year 191 182 168 152 130 105 70
10-year 178 157 130 113 101 85 63
RFI, HER2-, BCI (H/I)-High
10-year
5-year
9.4%
HR: 0.35 (0.15-0.81)
P = 0.047
Risk of Recurrence (%)
Years
No. at risk
5-year 161 143 124 109 91 76 49
10-year 181 161 138 123 108 96 59
B
DFI, HER2-, Unselected
10-year
5-year
3.8%
HR: 0.88 (0.68-1.15)
P = 0.366
Risk of Recurrence (%)
Years
No. at risk
5-year 352 323 286 255 215 176 116
10-year 359 318 267 234 206 178 118
DFI, HER2-, BCI (H/I)-Low
10-year
5-year
-1.7%
HR: 1.10 (0.75-1.62)
P = 0.612
Risk of Recurrence (%)
Years
No. at risk
5-year 191 182 166 151 129 104 69
10-year 178 157 129 112 100 84 62
DFI, HER2-, BCI (H/I)-High
10-year
5-year
10.3%
HR: 0.41 (0.18-0.91)
P = 0.047
Risk of Recurrence (%)
Years
No. at risk
5-year 161 141 120 104 86 72 47
10-year 181 161 138 122 106 94 56
C
DFS, HER2-, Unselected
10-year
5-year
3.1%
HR: 0.91 (0.71-1.18)
P = 0.492
Risk of Recurrence (%)
Years
No. at risk
5-year 352 323 286 255 215 176 116
10-year 359 318 267 234 206 178 118
DFS, HER2-, BCI (H/I)-Low
10-year
5-year
-1.3%
HR: 1.10 (0.76-1.60)
P = 0.623
Risk of Recurrence (%)
Years
No. at risk
5-year 191 182 166 151 129 104 69
10-year 178 157 129 112 100 84 62
DFS, HER2-, BCI (H/I)-High
10-year
5-year
8.5%
HR: 0.46 (0.22-0.98)
P = 0.045
Risk of Recurrence (%)
Years
No. at risk
5-year 161 141 120 104 86 72 47
10-year 181 161 138 122 106 94 56