Published in final edited form as:
Lancet Oncol. 2013 October ; 14(11): 1067–1076. doi:10.1016/S1470-2045(13)70387-5.

Prediction of late distant recurrence in estrogen receptor positive breast cancer patients: prospective comparison of the Breast Cancer Index (BCI), Oncotype DX recurrence score, and IHC4 in TransATAC

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SUMMARY

BACKGROUND—Greater than 50% of recurrences in estrogen receptor-positive (ER+) breast cancer occur after 5 years of adjuvant endocrine therapy. Biomarkers capable of improving the risk-benefit of extended adjuvant endocrine therapy for these late recurrences would be clinically valuable. We compared the prognostic ability of the Breast Cancer Index (BCI), Oncotype DX Recurrence Score (RS) and IHC4 for both early and late recurrence among patients with ER+, node negative (N0) disease within the ATAC clinical trial.

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Author's Contributions

DCS was the principal investigator. DCS, YZ, CS, MGE and PEG participated in all phases of this study, including design and writing of the biomarker proposal, submission to the ATAC Steering and Pathology Committees for approval, data collection, analysis and interpretation, and preparation of the manuscript. BS participated in data analysis and interpretation and writing of the manuscript. IS performed all of the statistical analyses and IS, MD and JC participated in study design, data analysis and interpretation, and preparation of the manuscript. AD, KS and EL participated in data collection and sample analysis. All authors have approved the contents of the manuscript.

Ethics Committee Approval

The conduct of this work was covered by an approval from the South-East London Research Ethics Committee and the Massachusetts General Hospital Institutional Review Board.

Conflicts of Interest

DCS and MGE are named inventors on a patent to use the Breast Cancer Index (BCI), HOXB13/IL17BR (H/I) and Molecular Grade Index (MGI) assays to predict breast cancer outcome. MD and JC declared that they have received grant support and lecture fees from Astrazeneca. PEG disclosed that he has received lecture fees from Novartis and Glaxo-Smith-Kline. MGE, CAS, YZ and BS are employees of bioTheranostics Inc. AD, KS, EL and IS have declared that they has no relevant conflicts of interest.

METHODS—BCI was performed from 1102 primary tumor samples from ER+ patients and two versions (BCI-C (primary) and BCI-L (secondary), based on cubic and linear combinations of the variables) were evaluated. RS and IHC4 values were previously derived. Prognostic discrimination for early (<5y) and late recurrence (5–10y) was assessed. To evaluate the ability of the biomarkers to predict recurrence beyond standard clinicopathological parameters, the likelihood-ratio chi-square (LR-$\Delta\chi^2$) was calculated from Cox proportional hazards models. The primary endpoint was distant recurrence (DR).

FINDINGS—In the primary analysis of 665 ER+ N0 patients, categorical BCI-C demonstrated significant differences in risk of DR over 10 years (P<0·0001). In the secondary analysis, BCI-L proved to be a much stronger predictor, and BCI-L, IHC4 and RS had significant prognostic performance for early DR (BCI-L, p<0·0002), while only BCI-L was significant for late DR (LR-$\Delta\chi^2$: 7·97, p=0·0048). For risk of early DR at 5 years, BCI-L classified 59% (390/665), 25% (166/665) and 16% (109/665) of patients with 1.3% (0.5% – 3.1%), 5.6% (2.9% – 10.5%) and 18.1% (12.0% – 27.0%) for low, intermediate and high risk, respectively. For risk of late DR at 10 years, BCI-L classified 61% (366/596), 25% (146/596) and 14% (84/596) of patients with 3.5% (2.0% – 6.1%), 13.4% (8.5% – 20.8%) and 13.3% (7.4% – 23.4%) for low, intermediate and high, respectively.

INTERPRETATION—While all three biomarkers predicted for early DR, BCI-L was the only significant prognostic for risk of late DR. The three BCI-L groups identified two risk populations for both early and late DR with 84% (556/665) of patients having low risk for early DR, and a smaller population (39%, 230/596) having high risk for late DR who may benefit from extended endocrine or other therapy.

FUNDING—Avon Foundation, National Institutes of Health, Breast Cancer Foundation, DOD Breast Cancer Research Program, Susan G. Komen for the Cure, Breakthrough Breast Cancer through the Mary-Jean Mitchell Green Foundation, Astrazeneca, NIHR Biomedical Research Centre at the Royal Marsden.

INTRODUCTION

Estrogen receptor-positive (ER+) breast cancer is a disease with a protracted risk of recurrence.[1,2] After five years of adjuvant tamoxifen, patients have an ongoing risk of disease recurrence and death for at least 15 years from diagnosis.[1] Long-term follow-up from pivotal up-front trials of adjuvant aromatase inhibitors, including the ATAC and BIG 1-98 studies, demonstrate an ongoing annual rate of recurrence of approximately 2% per year after initial therapy with greater than half of all recurrences occurring post 5 years of adjuvant endocrine therapy.[3–5] These findings underscore the need for consideration of extended adjuvant therapy and a biomarker that can guide this treatment decision-making process.

Multigene expression signatures studied over the past decade for assessment of recurrence risk in ER+ breast cancer rely primarily on the quantitative measurement of proliferation-related gene expression.[6–15] These multigene signatures, including Oncotype DX Recurrence Score (RS), are strong predictors of distant recurrence, but their prognostic performance diminishes when assessing risk beyond 5 years from diagnosis.[16,17] In contrast, predictors of late recurrence are not well characterized, and it is hypothesized that different mechanisms may be associated with early and late recurrences.[18,19] There is an unmet clinical need for biomarkers that identify patients who are adequately treated with only 5 years of endocrine therapy, and conversely, those patients at increased risk of late recurrence that may warrant extended adjuvant endocrine or other therapy. Biomarkers that have prognostic performance beyond clinicopathological factors for the prediction of late recurrence risk in ER+ breast cancer would have clinical utility.

We have previously developed and validated the breast cancer index (BCI) assay that consists of two independently developed gene expression biomarkers: molecular grade index (MGI) and HOXB13/IL17BR (H/I).[20,26] MGI, a 5-gene predictor that recapitulates tumor grade/proliferation, is highly prognostic in ER+ breast cancer patients.[21] H/I, which was developed independent of tumor grade/proliferation, is prognostic for early and late distant recurrences, and is predictive of extended adjuvant aromatase inhibitor benefit in early stage ER+ breast cancer patients.[12,22,23] Here, we evaluated the prognostic value of BCI for early and late distant recurrence (DR) in postmenopausal women with localized lymph node-negative (N0) breast cancer treated with either tamoxifen or anastrozole monotherapy in the ATAC trial, and compared its prognostic performance in matched patients to that of RS and IHC4.

METHODS

Patients and tumor samples

The TransATAC project was initiated in 2002 to establish a tissue bank from formalin-fixed paraffin-embedded (FFPE) primary tumor blocks from post-menopausal ER+ breast cancer patients from the monotherapy arms of the ATAC trial.[3,5] Archival tumor blocks were requested for patients except those known to be ER and PgR negative according to local tests, and those randomly assigned to the combination arm. RNA was extracted from FFPE blocks collected from the United Kingdom (79% of the total collection) by Genomic Health Inc. (Redwood City, CA, USA) for RS testing.[24] Samples with sufficient residual RNA available for BCI analysis were used in this study. The conduct of this work was covered by an approval from the South-East London Research Ethics Committee and the Massachusetts General Hospital Institutional Review Board.

Pathology and Analytic Methods

Immunohistochemical (IHC) analysis for ER, PgR, HER2 and Ki-67, tumor grade assessment, and IHC4 scores and Clinical Treatment Scores (CTS) were calculated as previously described.[25] CTS is a prognostic model using the classical variables of tumor size and grade, lymph node status, age and treatment.[25]

RNA extracts were available from a study of RS in the ATAC trial; RNA was extracted by GHI. BCI analysis of total RNA was conducted at bioTheranostics Inc. (San Diego, CA) blinded to clinical outcome. The pre-specified BCI genes, primer and probe sequences, RT-PCR and calculation of H/I and MGI were performed as previously described.[20–22] Two BCI models, cubic (BCI-C) and linear (BCI-L), (based upon cubic and linear combinations of the variables) were previously developed and validated in the tamoxifen-treatment arm and the non-treatment arm of the of the Stockholm trial, respectively and were pre-specified.[20,26,27] BCI has not been assessed in ER-negative or triple negative breast cancer patients. BCI and RS measure gene expression by quantitative real-time PCR, although they differ in the genes that they detect [6, 21]. IHC4 is a prognostic model that consists of the most informative combination of ER, PgR, HER2 and Ki-67 proteins, and this model has been validated in a Nottingham cohort independent of the TransATAC [25]. IHC4 differs from BCI in that it measures the protein expression of ER, PgR, HER2 and Ki-67, none of which are encoded by genes in the BCI assay.

The BCI score was linearly scaled to a final score (0–10). BCI low-, intermediate-, and high-risk groups were determined using pre-specified cut-points for each model: 5.0 and 6.4 for BCI-C and 5.0825 and 6.5025 for BCI-L.[26] RS risk groups were determined as previously described.[4] Three risk groups of IHC4 were determined using 2 cut-points that corresponded

to the 10-year distant recurrence rate of 10% and 20% in the TransATAC cohort, respectively.

Study objectives and endpoints

DR was the prospectively-defined primary endpoint, and refers to all recurrences to distant organs and excludes contralateral disease, locoregional and ipsilateral recurrences, and other second primary cancers; DRs experienced after locoregional recurrence were included as an event at the time of DR. The median follow-up was 9.97 years (interquartile range 8.50 to 10). Patients who died before DR were censored. All recurrences, breast cancer deaths (BCD) and overall survival (OS, time to death from any cause) were also evaluated as secondary endpoints. The primary analysis population was ER+ N0 patients, while the secondary analysis populations included ER+N0/HER2-negative and ER+ node-positive patients. The prospectively-defined primary study objective was to evaluate overall (0–10y) prognostic performance of the BCI-C model for DR in ER+ N0 patients. Secondary objectives were: 1) assessment of the prognostic performance of the BCI-L model and its components, H/I and MGI, for overall (0–10y), early (0–5y) and late (5–10y) DR; 2) comparative performance of BCI-L versus RS and IHC4.

Statistical analysis

A statistical analysis plan was approved by the ATAC/LATTE Steering Committee prior to study initiation. Early DRs were evaluated by censoring follow-up of all patients at 5 years post-diagnosis. Late DRs were evaluated within the subset of patients who remained DR-free for at least 5 years in order to assess whether the gene signature remained prognostic after its prognostic effect for early recurrence was removed. Likelihood ratio tests based on Cox proportional hazards (PH) regression models were used to test whether there was a significant difference between a reduced PH model based on CTS and a full PH model, including BCI, RS or IHC4. The improvement in prediction was quantified by the change in the likelihood ratio chi-square (LR-$\Delta\chi^2$) value, which measures the amount of the information added to the PH model by the gene signatures over CTS. As IHC4 was trained in a subset of TransATAC samples, sample splitting was performed as previously described to adjust for the potential overfitting. [25] Kaplan-Meier survival analysis was used to graphically present the survival curves of BCI's three pre-specified risk groups and the equality of the curves was tested with a log-rank test.

The risk of DR as a function of BCI as a linear covariate was calculated from Cox PH models for overall (0–10y), early (0–5y) and late (5–10y) DR. To compare the performance of BCI, RS and IHC4, the inter-quartile hazard ratio (HR) comparing the 75-percentile vs 25-percentile of the continuous scores of these biomarkers and the associated 95% confidence interval (CI) were estimated from Cox PH models. A two-sided p-value less than 0·05 was considered statistically significant. The RS was already studied in TransATAC and IHC4 was trained in a subset of these patients[24, 25]; the performance of RS and IHC as continuous scores was pre-specified and no multiple testing adjustment was planned or performed. Statistical analyses were performed with STATA version 12.1 (StataCorp, College Station, Texas, USA).

Role of Funding Source

This study was funded, in part, by grants from the Avon Foundation New York, the National Institutes of Health, Breast Cancer Foundation, the Department of Defense Breast Cancer Research Program, The Susan G. Komen for the Cure, Breakthrough Breast Cancer through the Mary-Jean Mitchell Green Foundation, the NIHR Biomedical Research Centre at the Royal Marsden Hospital and Astrazeneca. None of these sources of the funding sources had any role in the study design, the collection, analysis or interpretation of the data, or writing

of or the decision to submit this manuscript. The authors have not been paid to write this article by a pharmaceutical company or other agency. The BCI assays were undertaken at bioTheranostics by laboratory personnel who had no knowledge of treatment assignment or clinical outcome. The study biostatisticians (IV, JC) had the only direct access to the raw data. The corresponding author had full access to the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Characteristics of the Study Population

Values for RS, IHC4 and BCI were available for 915 women, of whom 665 were ER+ N0 (Supplemental Figure 1). Clinical characteristics of these 665 patients are listed in Table 1, along with characteristics of 561 ER+ N0 patients from the United Kingdom in the ATAC trial who were not included in this study. No significant differences were observed except that the non-TransATAC cohort had more well-differentiated tumors and fewer late DRs than the TransATAC patients. In N0 women, there were 106 recurrences, including 72 DRs and 7 local recurrences after mastectomy.

BCI and Risk of Overall (0–10y) DR

In the pre-specified primary analysis using the BCI-C model, Kaplan-Meier analysis (Figure 1A) demonstrated significant differences in absolute DR rates (P<0·0001) when analyzed according to pre-specified categorical BCI-C risk groups. The rates of DR at 10 years in the low, intermediate and high risk BCI-C groups were 6·8% (95% CI, 4·4% to 10·0%), 17.3% (95% CI, 12·0% to 24·7%), and 22·2% (95% CI, 15·3% to 31·5%), respectively. When adjusted for the effects of tumor size and grade, age and treatment (as determined by CTS), the HR between high- and low-risk BCI-C groups was 2·19 (95% CI, 1·19 to 4·02) and between the intermediate- and low-risk groups was 1·85 (95% CI, 1·04 to 3·28) (Figure 1A). BCI-C analyzed as a continuous variable was not significantly associated with overall (0–10y) risk of DR when adjusted for CTS (inter-quartile HR=1·39; 95% CI, 0·99 to 3·70; LR-$\Delta\chi^2$=3·70; *P*=0·054).

In the secondary analysis, assessment of pre-specified BCI-L revealed that this version was much more strongly associated with the overall risk of DR (inter-quartile HR=2·30; 95% CI, 1·62 to 3·27; LR-$\Delta\chi^2$=22·69; *P*<0·0001) than BCI-C when adjusted for CTS. Kaplan-Meier curves (Figure 1B) show clear differences in absolute DR rates according to pre-specified BCI-L risk groups (P<0·0001): the low-, intermediate- and high-risk BCI-L groups demonstrated rates of DR at 10 years of 4·8% (95% CI, 3·0% to 7·6%), 18·3% (95% CI, 12·7% to 25·8%), and 29·0% (95% CI, 21·1% to 39·1%), respectively. When adjusted for CTS, the HR between high- and low-risk BCI-L groups was 4·86 (95% CI, 2·58 to 9·17) and between the intermediate- and low-risk groups was 2·89 (95% CI, 1·55 to 5·40) (Figure 1B). The overall 10-year risk of DR increased linearly with increasing BCI-L (Supplemental Figure 2).

In the HER2-negative N0 subset, both BCI-C and BCI-L remained significantly associated with overall risk of DR with inter-quartile HR values of 1·65 (95% CI, 1·12 to 2·43; LR-$\Delta\chi^2$=6·61; *P*=0·0101) and HR= 2·49 (95% CI, 1·68 to 3·68; LR-$\Delta\chi^2$=21·9; *P*<0·0001), respectively. Kaplan-Meier curves of the pre-specified risk groups for both versions of BCI demonstrated distinct differences in absolute DR (Supplemental Figure 3).

Comparison of the prognostic performance of BCI-L to BCI-C indicated that unlike BCI-C, BCI-L was a significant predictor of risk of recurrence as a continuous variable, and the HR after adjustment with CTS was 2·19 versus 4.86 between high- and low-risk groups for BCI-

C and BCI-L, respectively. Thus, all subsequent analyses were performed utilizing BCI-L (henceforth referred to as BCI).

BCI and Risk of Early and Late DR

BCI was significantly associated with the risk of early (0–5y) DR (inter-quartile HR=2·77; 95% CI, 1·63 to 4·70; LR-$\Delta\chi^2$=15·5; *P*=0·00011, Table 2) when adjusted for CTS. Kaplan-Meier curves (Figure 2A) displayed significant differences in absolute DR rates at 5 years of 1·3% (95% CI 0·5% to 3·1%), 5·6% (95% CI 2·9% to 10·5%) and 18·1% (95% CI 12·0% to 27·0%) for the pre-specified BCI low-, intermediate- and high-risk groups, respectively. Although three risk groups were pre-specified, the results from the pre-specified Kaplan-Meier analysis revealed that the low and intermediate risk patients display similar rates of recurrence and together these patients constitute one group that is distinctly different from a second group that consists of high risk patients. A post-hoc Kaplan-Meier analysis revealed that there was little difference in DR at 5 years between the BCI low- and intermediate-risk groups that constituted 84% (556/665) of all patients (P1) with a combined 5-year rate of DR of 2·6% (95%CI 1·5% to 4·3%) (Figure 2A, Table 3). The BCI high-risk group (P2) that constituted 16% (109/665) of all patients had a 5-year rate of DR of 18·1%. When adjusted for CTS, the HR between the P1 and P2 was 4·61 (95% CI, 2·20 to 9·66).

For late (5–10y) recurrence, BCI was significantly associated with the risk of DR (HR=1·95; 95% CI, 1·22 to 3·14; LR-$\Delta\chi^2$=7·97; *P*=0·0048, Table 2) when adjusted for CTS. Kaplan-Meier curves (Figure 2B) displayed significant differences in absolute DR rates at 5 years of 3·5% (95% CI 2·0% to 6·1%), 13·4% (95% CI 8·5% to 20·5%) and 13·3% (95% CI 7·4% to 23·4%) for the BCI low-, intermediate- and high-risk groups, respectively. The results from the pre-specified Kaplan-Meier analysis of this period revealed that the intermediate and high risk patients display highly similar rates of recurrence and together these patients constitute one population that is distinctly different from a second population that consists of low risk patients. Additional post-hoc Kaplan-Meier analyses revealed that the DR of the BCI low-risk group (P3) that constituted 61% (366/596) of all patients with a 5-year rate of DR of 3·5% was substantially different from that of combined BCI intermediate- and high-risk groups (P4) that constituted 39% (230/596) of all patients and had a combined 5-year rate of DR of 13·4% (95% CI 9·3% to 19·0%) (Figure 2B, Supplemental Table 1). Adjusting for CTS, the HR between P3 and P4 was 2·94 (95% CI, 1·44 to 6·01). The risk of DR increased linearly with increasing BCI values for both early and late recurrence (Figure 3).

Given that the natural history of ER-positive HER2-positive breast cancer patients differs from that of ER-positive HER2-negative patients, we performed a subset analysis to assess whether the prognostic performance of BCI in the entire N0 ER-positive TransATAC cohort was unduly influenced by the inclusion of the subset of HER2-positive patients. In the HER2-negative N0 subset, BCI was significantly associated with the risk of both early DR (inter-quartile HR=3·26; 95% CI, 1·69 to 6·30; LR-$\Delta\chi^2$=13·53; *P*=0·00023, Table 2) and late DR (inter-quartile HR=2·12; 95% CI, 1·30 to 3·47; LR-$\Delta\chi^2$=9·45; *P*=0·0021, Table 2) and associated with distinct differences in absolute DR according to BCI risk groups (See Supplemental Figure 4). For both early and late recurrence the risk of DR increased with increasing BCI values (Supplemental Figure 5).

H/I, MGI, and Risk of Early and Late DR

H/I has been demonstrated to predict for late DR in the MA.17 cohort.[23] Thus, we assessed H/I and MGI, the individual components of BCI, for their prognostic value for the risk of early and late DR. For early recurrence, MGI and H/I added significant prognostic information (Table 4). However, for late recurrence only H/I provided additional information beyond standard clinicopathological factors (Table 4).

Comparison of BCI with IHC4 and RS

The correlation matrix among BCI-C, BCI-L, RS, IHC4 and CTS showed a strong correlation between the two versions of BCI and between IHC4 and RS, while both BCI-C and BCI-L had weak to moderate correlation (< 0.5) with RS, IHC4 or CTS (Supplemental Table 2). Kaplan-Meier curves of overall (0–10 year) distant recurrence for RS and IHC4 risk groups for both arms combined and separately for anastrozole and tamoxifen were shown in Figure 4. For both arms combined, BCI-low risk group had the lowest 10 year rate of distant recurrence (4.8%) as compared to RS (6.5%, 95% CI, 4.3% to 9.7%) and IHC4 (6.2%, 95% CI, 4.1% to 9.3%), while the BCI-high risk group had the highest rate of distant recurrence (29.0%, 95% CI, 21.1% to 39.1%) compared to RS (27.1%, 95% CI, 18.9% to 37.8%) and IHC4 (21.8%, 95% CI, 14.3% to 32.4%) (Figure 4).

The change in likelihood ratio χ^2 (LR-$\Delta\chi^2$) values were used to provide for a direct head-to-head comparison of BCI with IHC4 and RS. The relative prognostic performance of each biomarker varied depending upon the DR time frame (Table 2). For the early recurrence time frame, BCI, IHC4 and RS were all prognostic for DR in both univariate and multivariate analyses (Table 2). In all N0 patients, IHC4 was more prognostic than RS and BCI after adjusting for CTS. However, in the N0 HER2-negative patients, BCI and IHC4 demonstrated comparable prognostic performance that was superior to RS after adjusting for CTS. In the multivariate analysis of the 5–10y time frame, only BCI remained strongly prognostic in all N0 and N0 HER2-negative patients, while both IHC and RS were not prognostic in either population (Table 2). Similar results were observed considering all recurrences, BCD and OS as end points (Supplemental Table 3).

Although the primary analysis of this study centered on N0 patients, an analysis of node-positive patients revealed that BCI was also prognostic for DR in these patients (Supplemental Figure 6, log rank p=0.0045). Furthermore, a comparative analysis revealed that BCI, IHC4 and RS had highly similar prognostic performance, albeit less robust than that observed in the N0 subset (Supplemental Table 4).

DISCUSSION

We have shown that BCI has significant prognostic performance over 10 years for the prediction of individual risk of distant recurrence for hormone receptor-positive N0 patients in the TransATAC cohort. Examining clinically relevant time periods of 0–5y and 5–10y separately indicated that BCI may have the potential to impact two important decision points in the management of these patients. At baseline, BCI identified two apparently distinct groups of patients: a relatively small population (16%, 109/665) at high risk for early recurrence who do not benefit adequately from endocrine therapy alone, and should be considered for additional therapy (e.g. chemotherapy/other), and a large population (84%, 556/665) whose risk for early recurrence was sufficiently low that they may be considered adequately treated with endocrine therapy alone. For women disease-free after 5 years of therapy with either up-front adjuvant tamoxifen or up-front aromatase inhibitor, the two most common adjuvant therapies in clinical use, BCI also identified two distinct groups: a group of patients (39%, 230/596) at significant risk of late recurrence, and a second group (61%, 366/596) at very low risk of late recurrence (Table 3). For those at low risk after either up-front tamoxifen or aromatase inhibitor, BCI affords the option of no further systemic therapy of any sort. For those at high risk of recurrence after up-front adjuvant tamoxifen (patients with high H/I), we have recently shown that these patients do benefit from extended hormonal therapy with the aromatase inhibitor letrozole[23]. For those at high risk of recurrence after 5 years of up-front aromatase inhibitor, it is unclear whether these patients will benefit from extended adjuvant hormonal therapy or indeed to any systemic therapy. Approach to these patients will in part be guided by results from MA.17R and

NSABP B42 adjuvant trials (randomizing patients disease free after 5 years of adjuvant AI) to extended hormonal therapy or not. Alternatively these patients may also be candidates for experimental therapeutic approaches. BCI signature will likely help triage these patients appropriately.

BCI may be advantageous over other contemporary gene expression signatures, as the identification of two rather than three distinct risk groups in each time period potentially eliminates the less actionable intermediate risk category that can account for as many as 40% of ER+ patients.[28] In particular, BCI may be useful in the setting of late disease recurrence, as it may provide a much needed tool in identifying those patients who may be spared extended adjuvant endocrine therapy and its well-characterized adverse side effects. Previously, studies have demonstrated that clinicopathologic factors such as nodal status and tumor size are associated with a higher risk of late recurrence;[2,29] however, the results presented here represent a refinement, allowing for individualized assessment of late recurrence risk, and providing statistically significant improvement in prognostic strength above clinicopathologic factors. Recently disclosed preliminary studies have indicated that other gene expression-based assays (EndoPredict, PAM50) have prognostic performance for late recurrence beyond clinicopathological factors.[30,31] Taken together these data further validate the clinical use of molecular-based assays for the assessment of late disease recurrence risk.

Comparison of BCI to IHC4 and RS for overall 10 year risk reveals that all three biomarkers provided significant prognostic information. Within the 0–5 year time period, BCI and IHC4 performed nearly equally well and both provided greater prognostic information than RS (Table 2). During the 5–10 year time period, BCI was prognostic while IHC4 and RS were not. The limited prognostic performance of both IHC4 and RS for late recurrences is consistent with the data that RS and IHC4 were highly correlated (correlation = 0.71) while BCI had weak to moderate correlation with IHC4 or RS.

Analysis of H/I and MGI, the individual components of BCI, indicated that while each component was prognostic for early recurrence, only H/I was prognostic for late recurrence. The latter finding is consistent with results from a correlative study of the MA.17 trial in which H/I was prognostic for late recurrence.[23] Furthermore, the lack of prognostic strength of MGI for late recurrence is consistent with previous studies indicating that prognostic signatures relying primarily on measurement of proliferation-related gene expression have limited prognostic value for late recurrence.[16,17] Together these observations suggest that the H/I component of BCI provide additional information and unknown biological functionality beyond tumor proliferation; these H/I attributes may distinguish BCI from IHC4 and RS.

In addition, previous studies indicate that high expression of H/I is not only prognostic but also predictive of benefit of adjuvant endocrine-treatment.[23, 26] Thus, the marginal performance of BCI-C, which was trained in the endocrine-treated arm of the Stockholm trial, may have been confounded by the dual prognostic and endocrine-treatment predictive properties of H/I.[20] BCI-L, on the other hand, contains only additive functions of MGI and H/I and was trained in the untreated arm of the Stockholm trial in which clinical outcomes represented the natural history of breast cancer.[26] Findings reported here suggest that the BCI-L was spared any confounding effects of the endocrine treatment predictive properties of H/I, and as a result BCI-L was determined to be the optimal prognostic version of the combination of H/I and MGI.

There are strengths and limitations of our study. Strengths include the use of a standardized quantitative assay with methods and analyses prospectively-defined, and all assay data were

obtained blinded to study outcome or clinical parameters. An additional strength was the study's basis in a large contemporary prospective randomized clinical trial with relatively long follow-up. An important limitation was that in the primary analysis BCI-C was significantly prognostic as a categorical risk group variable (P<0.0001) but was not significantly prognostic as a continuous variable (P=0·054); thus, analyses relied on a secondary linear combination (BCI-L). However, both versions of BCI have been previously reported and validated.[20,26] In addition, our study included only post-menopausal patients with a follow-up period limited to 10 years. Thus, our findings may be limited to this population and this time frame. Also, an unintended selection bias may have applied to our N0 TransATAC cohort, as it consisted of a higher percentage of high grade tumors and a higher percentage of late DRs as compared with the N0 non-TransATAC patients. A limitation of this study is that Mammaprint has not been assessed in this cohort and thus this additional comparison could not be performed. Other limitations apply specifically to IHC4. Controversy exists over the variability and comparability of Ki-67 measurements in tissue samples but rigorous quality assurance standards were employed in this study in accordance with recommended guidelines for the measurement of IHC4.[32] Another limitation is that the IHC4 model was trained on the same data set, although the sample splitting procedure described previously adjusts for this potential overfitting[25]. Lastly, the lack of a pre-specified IHC4 categorical cut-point may limit the interpretation of its comparative prognostic performance in this study.

In summary, this study has confirmed the independent prognostic performance of BCI in post-menopausal hormone receptor-positive N0 breast cancer patients treated with tamoxifen. Furthermore, our results extend BCI's prognostic utility to include post-menopausal women treated with anastrozole, and it confirms BCI's ability to identify patients at increased risk for late recurrence. Future directions include further examination of the predictive performance of BCI for chemotherapy and extended adjuvant endocrine therapy benefit. From a clinical management view, our results suggest that BCI may have the potential to impact two important decision points in the management of post-menopausal ER+ N0 patients: first, at time of diagnosis; and second, at 5-year disease-free follow-up.

Systemic Review

We conducted a systematic review as part of the planning of this study. To identify previous biomarker studies of late recurrence in breast cancer, we conducted a search of Pubmed for reports published in English between Jan 1, 1980 and Dec 31, 2010. We used the terms "late recurrence" and "breast cancer". We retrieved 21 reports, of which we judged 17 to be most relevant.

Interpretation

As far as we are aware, this is the first published study to provide a comparative multi-biomarker analysis of early and late disease recurrence in a large randomized clinical trial of adjuvant hormonal therapy in post-menopausal ER+ breast cancer patients. Greater than 50% of all disease recurrences in ER+ breast cancer patients occur between 5 and 15 years after the time of diagnosis. Our biomarker identifies two distinct groups of patients, a group that consists of patients who are at low risk of recurrence and who might be adequately treated with adjuvant hormonal therapy alone, and another group that consists of patients who are vulnerable to a late recurrence and could be considered for adjuvant hormonal therapy or alternative therapy. Clinically our biomarker could allow many early stage ER+ breast cancer patients women to avoid unnecessary extended anti-hormonal treatment and could provide an important tool for aiding in the management of residual risk after 5 years of adjuvant hormonal treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the ATAC Trialists and Pathologists, the ATAC Pathology Sub-committee and the ATAC Steering Committee for their oversight. We thank Ranelle Salunga for technical expertise, and we are indebted to the women who participated in the ATAC trial. MD acknowledges support from the Royal Marsden NIHR Biomedical Research Centre.

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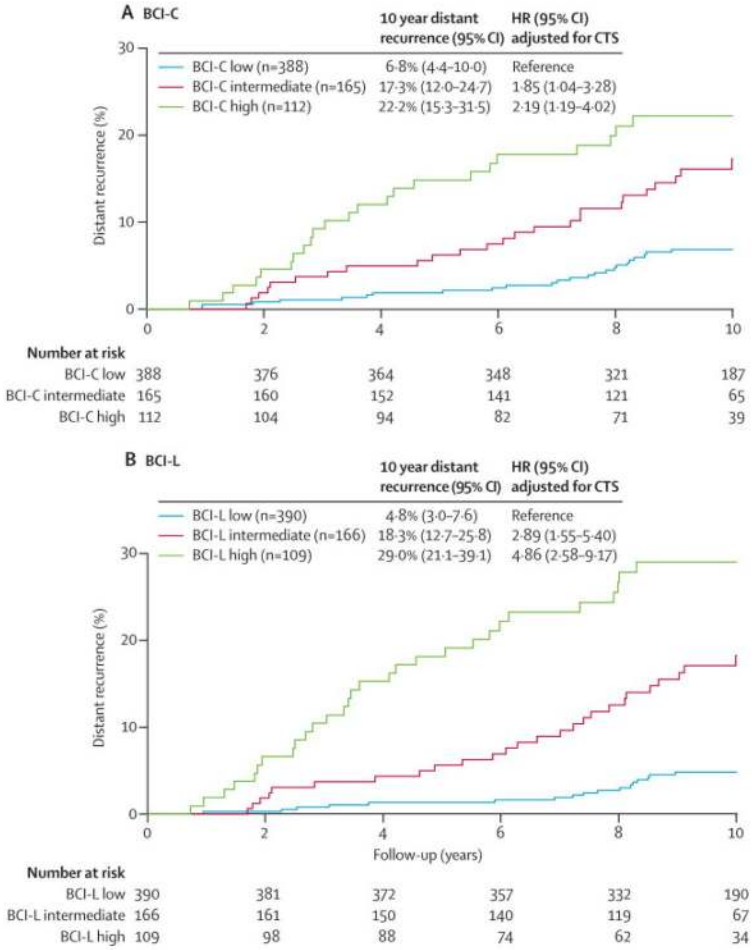

Figure 1.

Performance of pre-specified risk groups based on BCI-C and BCI-L for overall 10-year distant recurrences in all ER+N0 patients. A) BCI-C; B) BCI-L.

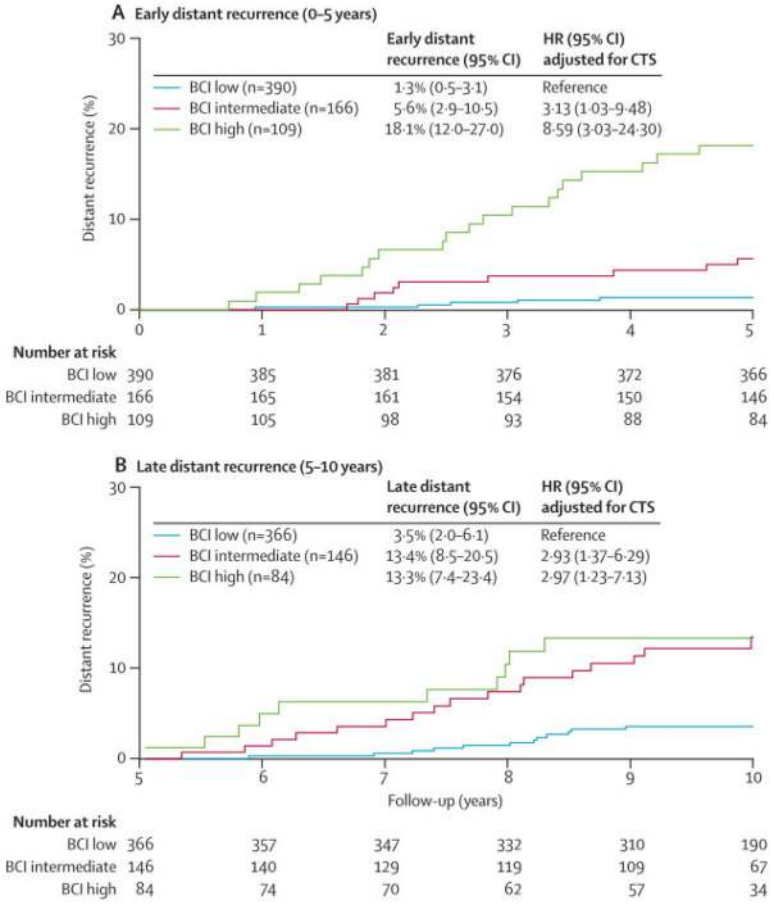

Figure 2.

Performance of BCI pre-specified risk groups for early and late distant recurrences in ER+ N0 patients. A) early 0–5 year distant recurrence; B) late 5–10 year distant recurrence. Population P1 refers to the pre-specified low and intermediate risk groups while P2, refers to the high risk group for early recurrence. P3 refers to the pre-specified low risk group, while P4 refers to the intermediate and high risk groups for late recurrence.

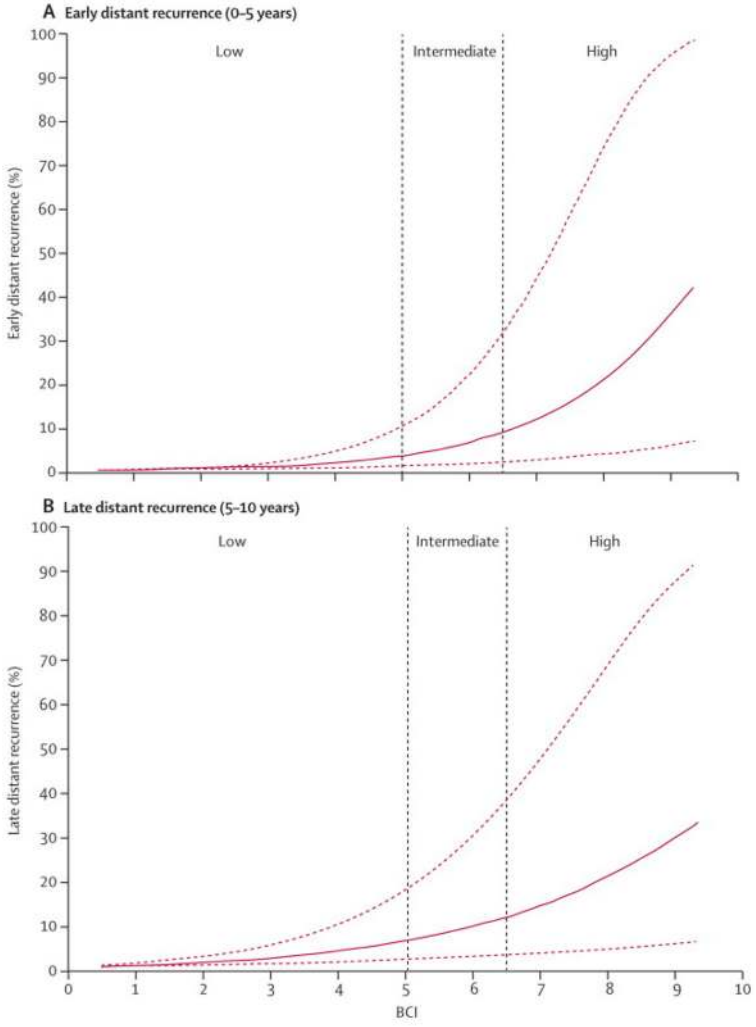

Figure 3.

Risk of early and late distant recurrence as a function of continuous BCI index in ER+ N0 patients. A) risk of early 0–5 year distant recurrence; B) risk of late 5–10 year distant recurrence. Vertical lines delineate the borders between the low, intermediate (Inter) and high pre-specified BCI risk groups.

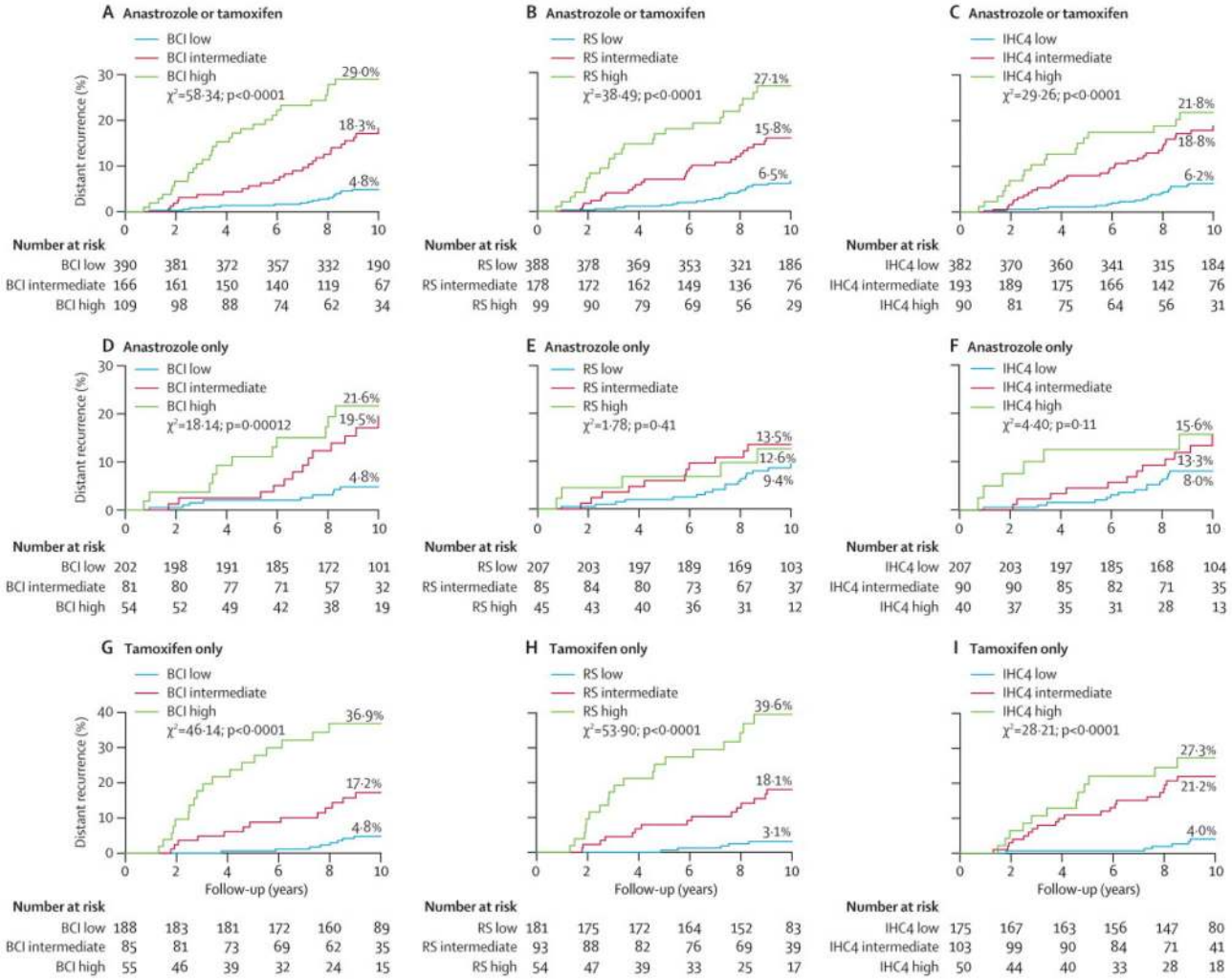

Figure 4.

Performance of the pre-specified risk groups of BCI and RS and the post-hoc determined categorical risk groups of IHC4 for overall 10-year distant recurrences in ER+ N0 patients, both arms combined and anastrozole (ANA) and tamoxifen (TAM) arm separately. A) BCI in both arms combined; B) RS in both arms combined; C) IHC4 in both arms combined; D) BCI in anastrozole arm alone; E) RS in anastrozole arm alone; F) IHC4 in anastrozole arm alone; G) BCI in tamoxifen arm alone; H) RS in tamoxifen arm alone; I) IHC4 in tamoxifen arm alone.

Table 1

Patient demographics and clinical characteristics in the present study and the broader population of N0 patients in single-agent arms of ATAC trial.

	N0 BCI cohort TransATAC (n=665)	N0 HER2neg BCI cohort TransATAC (n=597)	N0 UK patients Non-TransATAC* (n=561)	P value†
Age, mean (SD)	63.3 (8.1)	63.4 (8.0)	62.6 (7.8)	0.12
BMI, mean (SD)	27.1 (4.8)	27.2 (4.8)	26.8 (5.1)	0.28
Tumor size				0.13
<2 cm	486 (73.1%)	442 (74.1%)	432 (77.0%)	
2–3 cm	144 (21.7%)	125 (20.9%)	95 (16.9%)	
>3 cm	35 (5.2%)	30 (5%)	29 (5.2%)	
Unknown	0	0	5 (0.9%)	
Tumor grade				0.0051
Well	143 (21.5%)	138 (23.1%)	155 (27.6%)	
Moderate	395 (59.4%)	357 (59.8%)	300 (53.5%)	
Poor	127 (19.1%)	102 (17.1%)	78 (13.9%)	
Unknown	0	0	28 (5.0%)	
Radiotherapy				0.95
No	220 (33.1%)	189 (31.7%)	187 (33.3%)	
Yes	445(66.9%)	408 (68.3%)	374 (66.7%)	
Mastectomy				0.86
No	439 (66.0%)	404 (67.7%)	374 (66.7%)	
Yes	226 (34.0%)	193 (32.3%)	187 (33.3%)	
Treatment				
Anastrozole	337 (50.7%)	309 (51.8%)	285 (50.8%)	0.95
Tamoxifen	328 (49.3%)	288 (48.2%)	276 (49.2%)	
Distant Recurrence				
Early (0–5 years)	33 (5.0%)	21 (3.5%)	23 (4.1%)	0.56
Late (5–10 years)	39 (5.9%)	36 (6.6%)	12 (2.3%)	0.0022

*these are patients from the United Kingdom in the ATAC trial who do not have tumor blocks available for the translational study.

†comparison is between N0 TransATAC versus N0 Non-TransATAC cohorts. t tests were used for age and BMI, proportional test based on normal approximation was used for distant recurrence, all others used Fisher's exact test.

Abbreviations: ER, estrogen receptor; N0, node negative; HER2neg, human epidermal growth factor receptor 2 negative; BMI, body mass index; UK, United Kingdom

Table 2

Comparative prognostic performance for early and late distant recurrence of BCI, RS and IHC4 in all ER+ N0 patients and the ER+ N0 HER2-negative subset.

		Early Recurrence (0–5 Years)		Late Recurrence (5–10 Years)	
		HR* (95% CI)	LR-$\Delta\chi^2$ (P-value)	HR* (95% CI)	LR-$\Delta\chi^2$ (P-value)
UNIVARIATE					
BCI	N0	4.11 (2.52–6.70)	34.58 (<0.0001)	2.47 (1.59–3.83)	17.37 (<0.0001)
	N0 HER2neg	4.22 (2.32–7.64)	25.86 (<0.0001)	2.84 (1.80–4.48)	22.66 (<0.0001)
RS	N0	1.96 (1.60–2.41)	28.09 (<0.0001)	1.28 (0.95–1.72)	2.99 (0.21)
	N0 HER2neg	2.38 (1.61–3.53)	16.18 (<0.0001)	1.59 (1.09–2.31)	6.65 (0.014)
IHC4	N0	3.38 (2.39–4.78)	42.46 (<0.0001)	1.55 (1.06–2.26)	5.58 (0.022)
	N0 HER2neg	4.08 (2.26–7.36)	22.13 (<0.0001)	2.06 (1.29–3.28)	9.32 (0.0034)
MULTIVARIATE INCLUDING CTS					
BCI	N0	2.77 (1.63–4.70)	15.42 (<0.0001)	1.95 (1.22–3.14)	7.97 (0.0048)
	N0 HER2neg	3.26 (1.69–6.30)	13.65 (0.00023)	2.12 (1.30–3.47)	9.453 (0.0021)
RS	N0	1.80 (1.42–2.29)	18.48 (<0.0001)	1.13 (0.82–1.56)	0.48 (0.47)
	N0 HER2neg	1.93 (1.26–2.96)	8.37 (0.0041)	1.28 (0.87–1.88)	1.33 (0.28)
IHC4	N0	2.90 (2.01–4.18)	29.14 (<0.0001)	1.30 (0.88–1.94)	1.59 (0.20)
	N0 HER2neg	3.41 (1.83–6.39)	13.83 (<0.0001)	1.61 (0.98–2.66)	3.30 (0.086)

*HR was calculated as between the inter-quartile range of the continuous scores of each biomarker. Sample splitting was used to calculate HRs and χ^2 for IHC4.

Abbreviations: BCI, Breast Cancer Index; RS, OncotypeDX recurrence score; IHC4, four immunohistochemical markers (estrogen receptor, progesterone receptor, human epidermal growth factor 2, and Ki-67; HR, hazard ratio; LR-$\Delta\chi^2$, χ^2 value based on the likelihood ratio statistic; CTS, clinical treatment score; N0, node negative; HER2neg, human epidermal growth factor receptor 2 negative

Table 3

Absolute risk of early and late distant recurrence in clinically relevant subsets of ER+ N0 patients.

Early Recurrence (0–5 Years)		
BCI Risk Groups	**N (%)**	**Risk of Early DR at 5 Years (95% CI)**
low risk	390 (59%)	1.3% (0.5%–3.1%)
Intermediate risk	166 (25%)	5.6% (2.9%–10.5%)
high risk	109 (16%)	18.1% (12.0%–27.0%)

Late Recurrence(5–10 Years)		
Risk Subsets	**N (%)**	**Risk of Late DR at 10 Years (95% CI)**
low risk	366 (61%)	3.5% (2.0%–6.1%)
Intermediate risk	146 (25%)	13.4% (8.5%–20.8%)
high risk	84 (14%)	13.3% (7.4%–23.4%)

Table 4

Prognostic performance of H/I and MGI for early and late distant recurrence in ER+ N0 patients.

	Early Recurrence (0–5 Years)		Late Recurrence (5–10 Years)	
	HR*(95% CI)	LR-$\Delta\chi^2$ (P-value)	HR*(95% CI)	LR-$\Delta\chi^2$ (P-value)
UNIVARIATE				
MGI	3.15 (1.96–5.06)	24.27(<0.0001)	1.74 (1.16–2.62)	7.26 (0.0070)
H/I	2.42 (1.44–4.07)	10.90 (0.0010)	2.25 (1.38–3.66)	10.32 (0.0013)
MULTIVARIATE INCLUDING CTS				
MGI	2.10 (1.25–3.52)	8.41 (0.0037)	1.33 (0.86–2.06)	1.70 (0.19)
H/I	2.03 (1.20–3.41)	7.05 (0.0079)	2.02 (1.25–3.26)	8.17 (0.0043)

*HR was calculated as between the inter-quartile range of MGI and H/I.

Abbreviations: MGI, Molecular Grade Index; H/I, HOXB13/IL17BR gene expression ratio; HR, hazard ratio; LR-$\Delta\chi^2$, χ^2 value based on the likelihood ratio statistic; CTS, clinical treatment score; N0, node negative.