HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2024 March 08.

Published in final edited form as:

Clin Cancer Res. 2023 August 15; 29(16): 3101–3109. doi:10.1158/1078-0432.CCR-22-3753.

A multiparameter molecular classifier to predict response to neoadjuvant lapatinib plus trastuzumab without chemotherapy in HER2+ breast cancer

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Part of the data were presented orally at the following meeting:
Veeraraghavan J, Gutierrez C, De Angelis C, Wang T, Pascual T, Weigelt B, Galvan P, Rexer B, Forero A, Wolff AC, Nanda R, Storniolo AM, Krop IE, Goetz MP, Reis-Filho J, Hilsenbeck SG, Prat A, Osborne CK, Schiff R, Rimawi MF. A multiparameter classifier to predict response to lapatinib plus trastuzumab (LT) without chemotherapy in HER2+ breast cancer. 2020 American Society of Clinical Oncology. Journal of Clinical Oncology 2020 38:15_suppl, 1011-1011.

Conflicts of interests

C. Gutierrez reports holding Genetex stocks.

C. De Angelis reports personal fees (as consultant and/or speaker bureau) from Roche, Eli Lilly, GSK, Novartis, Pfizer, AstraZeneca; and Research grant to the institution from Novartis.

H. Nitta is an employee of Roche.

M. Kapadia is an employee of Roche and reports holding Roche stock.

A. Forero-Torres is an employee of Seagen.

I. E. Krop reports being on the advisory board of Bristol Myers Squibb, Daiichi Sankyo, Macrogenics, Genentech/Roche, Seagen, AstraZeneca; Data monitoring board of Novartis and Merck; Research funding to the institution from Genentech/Roche, Pfizer, Macrogenics.

R. Nanda reports being on the advisory boards of AstraZeneca, BeyondSpring, Fujifilm, Gilead, Infinity, iTeos therapeutics, Merck, OBI Pharma, Oncosec, Seagen; and Research funding from Arvinas, AstraZeneca, Celgene, Corcept Therapeutics, Genentech/Roche, Gilead/Immunomedics, Merck, OBI Pharma, Oncosec, Pfizer, Relay, Seagen, Sun Pharma, Taiho.

M. P. Goetz is the Erivan K. Haub Family Professor of Cancer Research Honoring Richard F. Emslander, M.D. and reports personal fees for CME activities from Research to Practice, Clinical Education Alliance, Medscape; Personal fees serving as a panelist for a panel discussion from Total Health Conferencing; Personal fees for serving as a moderator for Curio Science; Consulting fees to Mayo Clinic from ARC Therapeutics, AstraZeneca, Biovica, Biotheranostics, Blueprint Medicines, Eagle Pharmaceuticals, Lilly, Novartis, Pfizer, Sanofi Genzyme, Sermonix; and Research funding to Mayo Clinic from Lilly, Pfizer, Sermonix.

J. R. Nangia reports receiving clinical trial funding to the institution from Paxman Coolers Ltd.

B. Weigelt reports research funding from REPARE Therapeutics outside the scope of this study.

J.S. Reis-Filho reports receiving personal/consultancy fees from Goldman Sachs, Bain Capital, Repare Therapeutics, Paige.AI, Saga Diagnostics, and Personalis; membership of the scientific advisory boards of : VolitionRx, Repare Therapeutics, Paige.AI and Personalis; membership of the Board of Directors of Grupo Oncoclinicas; and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Daiichi Sankyo, Merck, and Astrazeneca; Stock options in Paige.AI; and stocks of Repare Therapeutics, outside the scope of this study.

A. Prat reports receiving honoraria from Pfizer, Novartis, Roche, MSD Oncology, Lilly, Daiichi Sankyo, Amgen, Guardant health; Consulting for Amgen, Roche, Novartis, Pfizer, Bristol-Myers Squibb, Boehringer, Puma Biotechnology, Oncolytics biotech, Daiichi Sankyo, AbbVie, AstraZeneca, Nanostring technologies (to the institution); Research funding to the institution from Roche, Novartis, Incyte, Puma Biotechnology; Stock and other ownership interests in Reveal Genomics; employment in Novartis (an immediate family member); Patents PCT/EP2016/080056 (*HER2 as a predictor of response to dual HER2 blockade in the absence of cytotoxic therapy*), WO/2018/096191 (*Chemoendocrine score (CES) based on PAM50 for breast cancer with positive hormone receptors with an immediate risk of recurrence*), and HER2DX filing; Travel and accommodation expenses from Daiichi Sankyo; and Other relationship with Oncolytics and Peptomyc S.L.

C. K. Osborne reports holding Genetex stocks and participation in AstraZeneca advisory boards.

R. Schiff receives/has received research funding from AstraZeneca, GlaxoSmithKline, Puma Biotechnology Inc, and Gilead Sciences (to the institution); Past ad hoc advisory committee member for Eli Lilly; Past consulting/advisory committee member for Macrogenics; Royalty from Wolters Kluwer/UpToDate (via institution).

M. F. Rimawi reports consulting for Seagen, AstraZeneca, Novartis, Genentech, Macrogenics; Research funding from Pfizer.

J. Veeraraghavan, C. Gutierrez, J. S. Reis-Filho, S. G. Hilsenbeck, A. Prat, C. K. Osborne, R. Schiff, M. F. Rimawi are also co-inventors in the pending patent application # PCT/US21/70543 (*Methods for breast cancer treatment and prediction of therapeutic response*) filed and owned by Baylor College of Medicine.

All remaining authors have declared no conflicts of interests

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Abstract

Background: Clinical trials reported 25–30% pathologic complete response (pCR) rates in HER2+ breast cancer (BC) patients treated with anti-HER2 therapies without chemotherapy. We hypothesize that a multiparameter classifier can identify patients with HER2 “addicted” tumors who may benefit from a chemotherapy-sparing strategy.

Patients and Methods: Baseline HER2+ BC specimens from TBCRC023 and PAMELA trials of neoadjuvant lapatinib+trastuzumab (plus endocrine therapy in ER+ tumors) were used. HER2 protein and gene amplification (ratio), HER2-enriched (HER2-E), and *PIK3CA* mutation status were assessed by dual gene protein assay (GPA), research-based PAM50, and targeted DNA-sequencing. GPA cutoffs and classifier of response were constructed in TBCRC023 using a decision tree algorithm, then validated in PAMELA.

Results: In TBCRC023, 72 BCs had GPA, PAM50, and sequencing data, of which 15 had pCR. Recursive partitioning identified cutoffs of HER2 ratio≥4.6 and %3+ IHC-staining≥97.5%. With PAM50 and sequencing data, the model added HER2-E and *PIK3CA* wild-type (wt). For clinical implementation, the classifier was locked as HER2 ratio≥4.5 and %3+ IHC-staining≥90% and *PIK3CA*-wt and HER2-E, yielding 55% and 94% positive (PPV) and negative (NPV) predictive values, respectively. Independent validation using 44 PAMELA cases with all three biomarkers yielded 47% PPV and 82% NPV. Importantly, our classifier's high NPV signifies its strength in accurately identifying patients who may not be good candidates for treatment de-escalation.

Conclusions: Our multiparameter classifier differentially identifies patients who may benefit from HER2-targeted therapy alone from those who need chemotherapy and predicts pCR to anti-HER2 therapy alone comparable to chemotherapy plus dual anti-HER2 therapy in unselected patients.

Keywords

HER2-positive breast cancer; HER2 levels and heterogeneity; *PIK3CA* mutations; PAM50 subtype; treatment de-escalation; molecular predictor

Introduction

Human epidermal growth factor receptor-2 (HER2) is amplified in 15–20% of breast cancer (BC). The paradigm in treating HER2+ BC has largely been treatment escalation by adding more HER2-targeted therapies to chemotherapy or adding other chemotherapy agents. This approach has significantly improved patient outcomes but comes with added clinical and financial toxicity. In the era of precision medicine, embracing a personalized approach driven by the molecular makeup of the tumor is rational and warrants exploring.

Preclinically, we and others have shown that multi-agent anti-HER2 therapy is superior to single agents in achieving potent HER family blockade, and when combined with endocrine therapy for estrogen receptor-positive (ER+) models achieved tumor eradication without chemotherapy [1–4]. We therefore postulated that a subset of patients with early-stage HER2+ BC may benefit from a de-escalated strategy of HER2-targeted therapy alone. This hypothesis was investigated in a series of 3 neoadjuvant chemotherapy-sparing trials TBCRC006 (NCT00548184), TBCRC023 (NCT00999804) and PAMELA (NCT01973660) [5–7]. Overall, in these trials, 25–30% of BC patients achieved pathologic complete response (pCR) with lapatinib+trastuzumab alone, plus endocrine therapy if ER+ [5–7]. These results suggest that a subset of HER2+ BCs is addicted to HER2 and that identifying such patients would permit a de-escalation approach. These patients should be accurately distinguished from the patients who need chemotherapy, which requires the development of clinically useful biomarkers using specimens from trials conducted without the confounding effect of chemotherapy.

We previously showed using baseline specimens from our neoadjuvant TBCRC006 study that a combinatorial biomarker of high HER2 gene levels (HER2 FISH ratio ≥4 and/or copy number (CN) ≥10) and intact PI3K pathway is associated with pCR from HER2-targeted therapy alone [8]. While the current ASCO/CAP guidelines define HER2 positivity as

FISH ratio ≥2, HER2 CN ≥6.0 for adding trastuzumab to chemotherapy [9], our findings suggest the need for higher HER2 levels to identify patients with highly HER2-addicted tumors that are likely to benefit from HER2-targeted therapy alone. Further, heterogeneous intra-tumoral HER2 levels are associated with worse patient outcome and resistance to HER2-targeted therapy, including trastuzumab-containing regimens [10–15]. It is therefore crucial to consider HER2 intra-tumoral heterogeneity (ITH) for treatment decisions. The HER2-enriched (HER2-E) subtype has been reported to be associated with greater response to anti-HER2 therapy in combination with chemotherapy [16, 17]. In the chemotherapy-free setting of PAMELA, a favorable response to lapatinib+trastuzumab was observed in the HER2-E subtype [6], which was further validated in a combined analysis with TBCRC023 and TBCRC006 specimens [18].

We therefore hypothesized that a multiparameter classifier combining HER2 expression, measured at the DNA and protein levels, together with HER2 ITH, HER2 signaling by intrinsic subtyping, and PI3K pathway status may predict sensitivity to HER2-targeted therapy alone and be a gateway for successful treatment de-escalation. Here we report the development of a multiparameter molecular classifier using baseline specimens from TBCRC023 as the training cohort and PAMELA as the independent validation cohort.

Methods

Study design and patients

Baseline specimens from two chemotherapy-sparing neoadjuvant trials, TBCRC023 (NCT00999804) [7] and PAMELA (NCT01973660) [6] were used. The study design and clinical results of both trials were previously reported [6, 7]. In TBCRC023, a multicenter randomized trial conducted through the Translational Breast Cancer Research Consortium (TBCRC), eligible participants with stage II or III HER2+ BC were enrolled to receive either 12 or 24 weeks of lapatinib+trastuzumab, and endocrine deprivation therapy if the tumor was hormone receptor-positive (HR+) (Supplementary Figure S1A). The overall pCR rate was 19.4%. Samples were available from 124 participants evaluable for response. In the PAMELA trial, participants with stage I-III HER2+ BC were treated for 18 weeks with an identical regimen (Supplementary Figure S1A). The overall pCR rate was 30.9%. Samples were available from 149 evaluable participants. Baseline specimens from patients deemed non-evaluable for response were excluded from all biomarker analyses (Supplementary Table 1). For both trials, institutional review board and scientific committee approvals were obtained at the lead site and other participating sites. Written informed consent was obtained from all patients. The studies were performed in compliance with the Declaration of Helsinki and all applicable US Federal and international regulations and ethical standards.

Tumor HER2 GPA analysis

Evaluable baseline specimens were analyzed using the dual HER2 Gene Protein Assay (GPA) for a quantitative assessment of HER2 gene levels by colorimetric in situ hybridization and HER2 protein level and ITH by immunohistochemistry (IHC) on the same slide, as previously described [19]. The GPA assay was performed on the BenchMark ULTRA IHC/ISH System, according to manufacturer's recommendation at

Roche Diagnostics, and the stained slides were reviewed and scored by the primary study pathologist at Baylor College of Medicine, blinded to clinical outcome. If the specimen was homogeneous for HER2 protein staining intensity with 100% 3+ staining, HER2 gene and protein levels were scored by counting *HER2* gene and chromosome 17 (chr17) signals in 40 representative epithelial cancer cell nuclei in the invasive region. If the specimen was heterogeneous for HER2 protein intensity with areas of 3+, 2+ and 0/1+ staining, the % of tumor cells representing each intensity level was estimated and 40 representative nuclei were counted in each of the 3+, 2+, and 0/1+ regions for *HER2* gene and chr17 signals. *HER2* gene ratio and CN were then computed by weighted average of the total signal in all staining areas. To add further validity to the scores and account for inter-reader variation, a second expert pathologist from Roche/Ventana Medical Systems was employed as a consultant to review all slides and score *HER2* gene ratio, CN, and protein. For slides where the readings were discordant between the two pathologists, the primary pathologist after re-review either continued with the initial read or changed the read if the primary pathologist agreed with the read of the second pathologist, which are hereafter referred as "*reassessed GPA data*".

Tumor HER2-E status analysis

Intrinsic molecular subtypes were determined with the research-based PAM50 predictor as previously reported [6]. Briefly, RNA extracted from baseline formalin-fixed paraffin-embedded (FFPE) specimens was used to measure the expression of 50 subtype predictor genes and five housekeeping genes using the nCounter platform (Nanostring technologies, Seattle). PAM50 subtyping was performed at the Translational Genomics and Targeted Therapies in Solid Tumors at IDIBAPS (Barcelona, Spain) by investigators who were blinded to the clinical data.

Targeted sequencing analysis

DNA samples extracted from evaluable formalin-fixed, paraffin-embedded baseline tumor specimens and matched normal DNA extracted from blood, when available, were subjected to massively parallel sequencing using MSK-IMPACT targeting 468 cancer-related genes, as previously described [20, 21]. Sequencing data were analyzed as previously described [21, 22], and identified amplifications, homozygous deletions and hotspot mutations as defined by Chang et al. [23] were mapped to the PI3K pathway, or the PI3K pathway plus receptor tyrosine kinases (RTKs) based on the oncogenic signaling pathways described by The Cancer Genome Atlas [24].

Data analysis and interpretation to construct the molecular classifier and evaluate its performance

As pre-specified, the TBCRC023 cohort was used as a training set to develop, refine, and lock the molecular classifier. The PAMELA cohort was used as the validation set to independently validate the locked algorithm. Analysts and investigators at Baylor College of Medicine were blinded to outcomes of the PAMELA cohort until after the cases were classified. The classifier was developed on TBCRC023 cases in two steps. First, using recursive partitioning, cases with GPA data (N=115) were used to determine the optimal HER2 gene ratio, CN and protein cutoffs to predict response as defined by pCR. Then, recursive partitioning was used to determine what to add to the indicator variable, based

on a composite of the GPA cut-offs, to construct a classifier on TBCRC023 cases with complete GPA, PAM50 and MSK-IMPACT data (N=72). Variables considered in various combinations for addition to the optimized GPA indicator included: HER2-E status, ER/PR status, *PIK3CA* mutation status, mutation status of any other members of the PI3K pathway, mutation status of RTKs [24], and *NF1* mutation status. Tree models and cut-offs were computed and explored using R (v3.6.2 – v4.1.2, RRID:SCR_006442) with packages rpart (v4.1–13 – v4.1–15, RRID:SCR_021777) for selection, and vtree (v4.0.0 - v5.4.6, RRID:SCR_023458) for visualization.

Data availability

The targeted sequencing data for all evaluable clinical specimens are deposited in the National Center for Biotechnology Information (NCBI) Sequence Reads Archive (SRA) with the accession ID: PRJNA962687.

Results

Cohort characteristics

Of the total 273 evaluable HER2+ BC patients, 70 (25.6%) achieved pCR in the breast. TBCRC023 and PAMELA cases were similar in age and nodal status, but TBCRC023 cases were more often pre-menopausal, had larger tumors, which were more often HR+ and less often HER2-E (Table 1). In the TBCRC023 cohort, of the 124 samples from evaluable participants, GPA data was available for 115, of which PAM50 subtyping was available for 85 specimens, with DNA sequencing data available for 72 of them (Supplementary Figure S1B). In the PAMELA cohort, of the 149 samples from evaluable participants, GPA data was available for 91 specimens, all of which also had PAM50 subtype information. Of these, DNA sequencing data was available for 44 specimens (Supplementary Figure S1B). Within each trial, the cohort of cases with complete data did not differ from the cohort of all evaluable cases (Table 1).

HER2 GPA analysis and response

The distribution of HER2 gene and protein levels were remarkably similar across the TBCRC023 and PAMELA cohorts, with a high correlation (r=0.64, $P<0.001$) between the two variables (Figure 1). Recursive partitioning analysis in the TBCRC023 cohort identified the optimal cutoffs for HER2 protein defined as percentage of tumor cells with 3+ staining and *HER2* gene ratio to be 97.5% and 4.6, respectively (Supplementary Figure S2A). Importantly, all TBCRC023 cases that achieved pCR, except for one, showed nearly 100% tumor cells with 3+ HER2 staining. Similarly, all but 5 PAMELA cases that achieved pCR harbored nearly 100% 3+ HER2 staining (Supplementary Figure S2B).

HER2-E subtype analysis

In the TBCRC023 cohort, HER2-E subtype represented 60% of the cases (51/85), followed by normal-like (12/85 [14%]), basal-like (11/85 [13%]), luminal B (7/85 [8%]), and luminal A (4/85 [5%]) (Supplementary Figure S3). Likewise, in the PAMELA cohort, HER2-E subtype represented the majority of cases (100/149 [67%]), followed by luminal A (22/149 [15%]), luminal B (15/149 [10%]), basal-like (9/149 [6%]), and normal-like (3/149 [2%])

(Supplementary Figure S3). We previously reported that the HER2-E subtype was associated with higher pCR rate in both cohorts [18].

PIK3CA (and PI3K pathway) mutation analyses

The prevalence and distribution of hotspot/pathogenic genomic alterations in the *PIK3CA* gene, and in genes affecting the PI3K pathway or PI3K pathway+RTKs, as defined by Sanchez-Vega et al[19] was largely similar across the two cohorts. Specifically, the incidence of *PIK3CA* hotspot/pathogenic genetic alterations was 27% and 29% in the TBCRC023 and PAMELA cohort, respectively, with mutations in the helical and kinase domain being predominant (Figure 2 and Supplementary Table 2). Additional pathogenic alterations detected in PI3K pathway and RTK genes are listed in Supplementary Table 3 and 4.

Decision tree algorithm to construct the multiparameter molecular classifier of response

As pre-specified, we used a decision tree algorithm to construct the *empirical molecular classifier* of response (by pCR) using the TBCRC023 cohort as a training set. Of the 72 TBCRC023 cases with information for all three biomarkers (HER2 GPA, PAM50 subtyping, and *PIK3CA* mutation status), there were 15 pCRs and 57 non-pCRs. Previously identified optimal cut-offs for GPA ratio and % 3+ HER2 staining were used to create a composite indicator variable which turned out to be the first splitting parameter (HER2 GPA ratio ≥ 4.6 and % 3+ HER2 staining ≥ 97.5%), which identified 14 of the 15 pCRs and retained 24 of the 57 non-pCRs (Figure 3A). The algorithm then added HER2-E subtype as the next splitting parameter, which correctly identified 12 pCRs and retained 15 non-pCRs. The model then added wild-type *PIK3CA* as the final decision node, which identified 12 pCRs and retained only 9 non-pCRs. For purposes of clinical implementation, the optimal HER2 GPA cutoffs were rounded to gene ratio ≥ 4.5 and % 3+ HER2 staining ≥ 90%. The final molecular classifier was locked as HER2 gene ratio ≥ 4.5 + % 3+ HER2 staining ≥ 90% AND HER2-E subtype AND wild-type *PIK3CA* (*hereafter referred as "molecular classifier"*, Figure 3B).

Performance of the classifier in TBCRC023 training cohort

The re-substitution performance of the locked molecular classifiers was first evaluated in the TBCRC023 training cohort using 72 specimens with data for all 3 biomarkers. The empirical molecular classifier correctly predicted 12 of 15 pCRs (80% sensitivity) and 48 of the 57 non-pCRs (84% specificity), with a positive predictive value (PPV) of 57% and negative predictive value (NPV) of 94% (Table 2). The molecular classifier correctly predicted 12 of the 15 pCRs (80% sensitivity) and 47 of the 57 non-pCRs (83% specificity), with a PPV of 55% and NPV of 94%.

We then evaluated the performance of our classifier in correctly identifying patients who may not respond to HER2-targeted therapy alone, using cases that had data for some but not all three biomarkers based on which they could, however, be predicted as non-pCRs either due to low HER2 gene and protein levels, non-HER2-E status, or mutant *PIK3CA*. In this analysis, our molecular classifier correctly identified 27 of the 29 non-pCRs, yielding a NPV of 93% (Supplementary Table 5). Finally, we assessed the performance of our molecular classifier in the cohort of specimens with reassessed GPA data. In the cohort with data

for all 3 biomarkers, our molecular classifier correctly identified 11 of the 15 pCRs (73% sensitivity) and 47 of the 57 non-pCRs (83% specificity), with 52% PPV and 92% NPV, while in the partial cohort, 29 of the 31 non-pCRs were correctly identified, yielding 94% NPV (Supplementary Figure S4, Table 3).

Independent validation of the classifier in PAMELA cohort

An independent validation in the PAMELA cohort using 44 specimens with data for all 3 biomarkers, showed that the empirical molecular classifier correctly predicted 8 of 13 pCRs (62% sensitivity) and 23 of the 31 non-pCRs (74% specificity), with a PPV of 50% and NPV of 82%, while the molecular classifier correctly predicted 8 of the 13 pCRs (62% sensitivity) and 22 of the 31 non-pCRs (71% specificity), with a PPV of 47% and NPV of 82% (Supplementary Figure S5A, Table 2). Further, using specimens that had partial biomarker data based on which they could be predicted as non-pCRs either due to low HER2 gene and protein levels, non-HER2-E status, or mutant *PIK3CA*, our classifier correctly identified 52 of the 64 non-pCRs, yielding a NPV of 81% (Supplementary Table 5). Finally, assessing the performance of our molecular classifier using specimens with reassessed GPA data, our classifier correctly identified 9 of the 14 pCRs (64% sensitivity) and 24 of the 31 non-pCRs (77% specificity) in the cohort with data for all 3 biomarkers, yielding 56% PPV and 83% NPV, while in the partial cohort, 52 of the 63 non-pCRs were correctly identified, yielding 83% NPV (Supplementary Figure S5B, Table 3). Exploratorily, we computed the classifier's performance by HR-status in the combined cohort of TBCRC023+PAMELA, though we did not perform a formal comparison due the small sample size and ad-hoc nature of the analysis. The results are summarized descriptively in Supplementary Table 6. There is no evidence that the classifier performs differently based on the HR status.

Discussion

Here, we report the development and independent validation of a multiparameter molecular classifier that identifies, based on tumor biology, patients who may benefit from a chemotherapy-sparing neoadjuvant regimen of dual HER2-targeted therapy alone. The multiple components of our classifier enhance its accuracy in identifying the highly HER2-dependent tumors by excluding most non-eligible cases that eluded one step of the algorithm in the subsequent steps. In line with the pCR rate observed in our neoadjuvant trials of dual HER2-targeted therapy, without chemotherapy [5, 7, 25], the WSG-ADAPT study of neoadjuvant pertuzumab+trastuzumab with or without paclitaxel for HER2+/HR- disease recently reported 34% pCR in the chemotherapy-sparing arm [26]. Importantly, irrespective of the treatment arm, achievement of pCR in WSG-ADAPT strongly correlated with improved invasive disease-free survival at 5 years, suggesting that pCR is a meaningful indicator of outcome in both settings and that a subgroup of patients may benefit from chemotherapy-sparing therapy without compromising long-term outcome [26, 27]. With the promise of de-escalation established in HER2+ BC, the emphasis is now on development of biomarkers for accurate patient selection.

This study highlights the need for high and homogeneous HER2 gene and protein levels and absence of PI3K pathway deregulations to benefit from a chemotherapy-sparing regimen

(Supplementary Figure S6), which further support the findings from our recent TBCRC006 correlative analysis [28]. Anti-HER2 therapy in combination with chemotherapy is shown to be associated with inferior response rates in patients with *PIK3CA* mutations in both neoadjuvant [29, 30] and adjuvant [31] settings. Further, analysis of PAMELA specimens revealed HER2-E subtype as an important predictor of sensitivity to dual HER2 blockade alone in patients with HER2+ BC[25]. Along this line, the neoadjuvant WSG-ADAPT study showed the association of treatment benefit with PAM50 subtype, with the HER2-E tumors deriving the greatest benefit, and no pCR in patients with low HER2 expression [26]. The significance of spatial HER2 ITH in HER2+ BC has been highlighted by several recent studies, including its impact on clinical outcomes and as a mechanism of resistance to HER2-targeted therapy [10–13]. In this study, all cases that achieved pCR, except 1 in TBCRC023 and 5 in PAMELA, showed HER2 3+ staining in about 100% tumor cells, emphasizing the importance of HER2 ITH in the chemotherapy-free setting and that it may be associated with inferior response to HER2-targeted therapy alone. In the neoadjuvant KRISTINE trial, more locoregional progression and lower event-free survival (EFS) rates were observed in the TDM1+pertuzumab arm, potentially attributable to the ITH in HER2 expression [32]. Indeed, retrospective analysis revealed lower pCR rates in patients with variable HER2 protein staining compared to those with homogeneous HER2 levels [14]. Similarly, a phase II study of TDM1+pertuzumab reported lack of pCR in patients with tumors showing HER2 heterogeneity [33].

Our classifier is thus built to identify the highly HER2-addicted tumors by assessing the tumor's functional dependence on HER2 at multiple levels: high HER2 gene and protein levels, lack of ITH in HER2 protein expression, HER2 signaling, and the absence of deregulations in HER2/downstream PI3K signaling pathway, which, if present, may undermine the benefit of HER2 blockade by transmitting alternative survival signals inside the cell. While our classifier was developed and validated using cohorts of specimens from patients who received lapatinib plus trastuzumab, a combination that is not part of current neoadjuvant therapy, the biological foundation of our classifier and its value and potential in predicting response to the newer more potent anti-HER2 agents of the current era continues to hold true. Our classifier predicts pCR rates in this selected group comparable to that of chemotherapy plus dual anti-HER2 therapy in unselected patient population. It accurately predicted pCR with a sensitivity of 80% and 62% in the training and validation cohorts, respectively. Importantly, the negative predictive value was high in both cohorts, signifying that it can also accurately identify patients whose tumors are not HER2 addicted and therefore may not be good candidates for treatment de-escalation, which is crucial for a safe de-escalation approach. The NPV was above 80% in both cohorts and continued to be high in cohorts that had data for some but not all biomarkers. These findings demonstrate the promise and performance of our classifier and warrant further validation in a prospective clinical trial in early stage HER2+ BC. If validated prospectively, our classifier may function as a molecular triaging tool to safely and appropriately select patients with HER2+ BC for treatment de-escalation. This biology-based approach may provide a paradigm shift with meaningful reduction of the treatment-associated financial and clinical toxicity as well as improvement in the quality of life. Moreover, the biomarkers that our classifier encompasses may help identify and triage patients for a personalized treatment using existing or newer

anti-HER2 regimens and agents, like using newer antibody-drug conjugates for tumors with HER2 ITH or low levels of HER2 expression or signaling. In recent years, alternative strategies for the early identification of patients who may respond better to neoadjuvant HER2-targeted therapy have also been explored. These include reports of correlation between lower relapse rate and perioperative reduction in Ki67% [34], and correlation between pCR and early change in tumor maximum standardized uptake corrected for lean body mass (SULmax) on 18F-labeled fluorodeoxyglucose positron emission tomography-computed tomography [35].

There are a few limitations in this study. Apart from the relatively small patient cohorts, we were also limited by tumor tissue availability for genomic analysis. While mutation status of *PIK3CA* gene emerged as a statistically significant predictor of response, the significance of alterations in additional components of the PI3K pathway and RTKs, and other rare or private alterations could not be statistically established given the small cohort size. The sensitivity and specificity of the classifier's performance was slightly lower in the validation cohort. These limitations, however, are outweighed by the evident strengths of this study, including being the first study to utilize completely unique cohorts of tumor specimens from different chemotherapy-sparing neoadjuvant trials to develop and independently validate a multiparameter molecular classifier of response to HER2-targeted therapy alone, without chemotherapy, based on the biology of the tumor. Our study addresses one of the key pieces in the de-escalation puzzle, accurate patient selection, and may inspire several future clinical studies to make effective treatment de-escalation possible. Finally, given the pioneering nature of these trials, most patients received standard of care adjuvant therapy, including chemotherapy, and therefore the long-term outcome for these patients on a chemotherapy-sparing regimen and its correlation with pCR and our molecular classifier could not be assessed. While this may seem like a current limitation, it also highlights the significance and novelty of our current study to build a molecular classifier, which will enable triaged prospective validation studies in the near future, including for the assessment of long-term outcome in a chemotherapy-sparing setting.

Treatment de-escalation in HER2+ BC has garnered much enthusiasm in the recent years with many trials, including ours [5, 7, 25], demonstrating the benefit of de-escalated therapy, including shorter duration of targeted therapy [36], less-intensive chemotherapy [32, 37–40], or complete omission of chemotherapy [5, 7, 25, 26, 41]. Our molecular classifier carries substantial promise as a precision medicine tool for treatment de-escalation, and various personalized treatment approaches. Planned and ongoing efforts are further exploring its utility in these settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Financial support:

This work was supported by the DOD breast cancer research program breakthrough awards CDMRP W81XWH-17-1-0579 (to M.F.R) and W81XWH-17-1-0580 (to R.S); Breast Cancer Research Foundation grants BCRF-19-145, BCRF-20-145, BCRF-21-145, BCRF-22-145 (to R.S and C.K.O) and BCRF-21-135 (to J.S.R.-F.

and B.W.), NIH SPORE Grants P50 CA186784 (to R.S, C.K.O, S.G.H, and M.F.R) and NIH/NCI P50 CA247749 01 (J.S.R-F. and B.W.); and Cancer Center Grants P30 CA125123 (BCM) and P30 CA008748 (MSKCC). We are grateful for the funding support to the TBCRC from The Breast Cancer Research Foundation and Susan G. Komen.

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Translational Relevance:

Past trials have reported an overall pathologic complete response (pCR) rate of 25–30% in HER2+ breast cancer (BC) patients treated with dual anti-HER2 therapy without chemotherapy, suggesting that a subset of HER2+ BCs is addicted to HER2 and that identifying such patients would permit a de-escalation approach. Accurately distinguishing these patients from those who need chemotherapy requires the development of clinically useful biomarkers. Here we successfully developed and independently validated a multiparameter molecular classifier consisting of HER2 gene levels and intra-tumor heterogeneity, intrinsic subtype, and *PIK3CA* status, which predicts response to anti-HER2 therapy alone without chemotherapy. Our classifier also holds a high negative predictive value, which signifies its accuracy in identifying patients who may need chemotherapy or other targeted therapies. Our classifier predicts pCR rates comparable to that of chemotherapy+anti-HER2 therapy in unselected patient population and carries substantial promise as a precision medicine tool for treatment de-escalation and personalization.

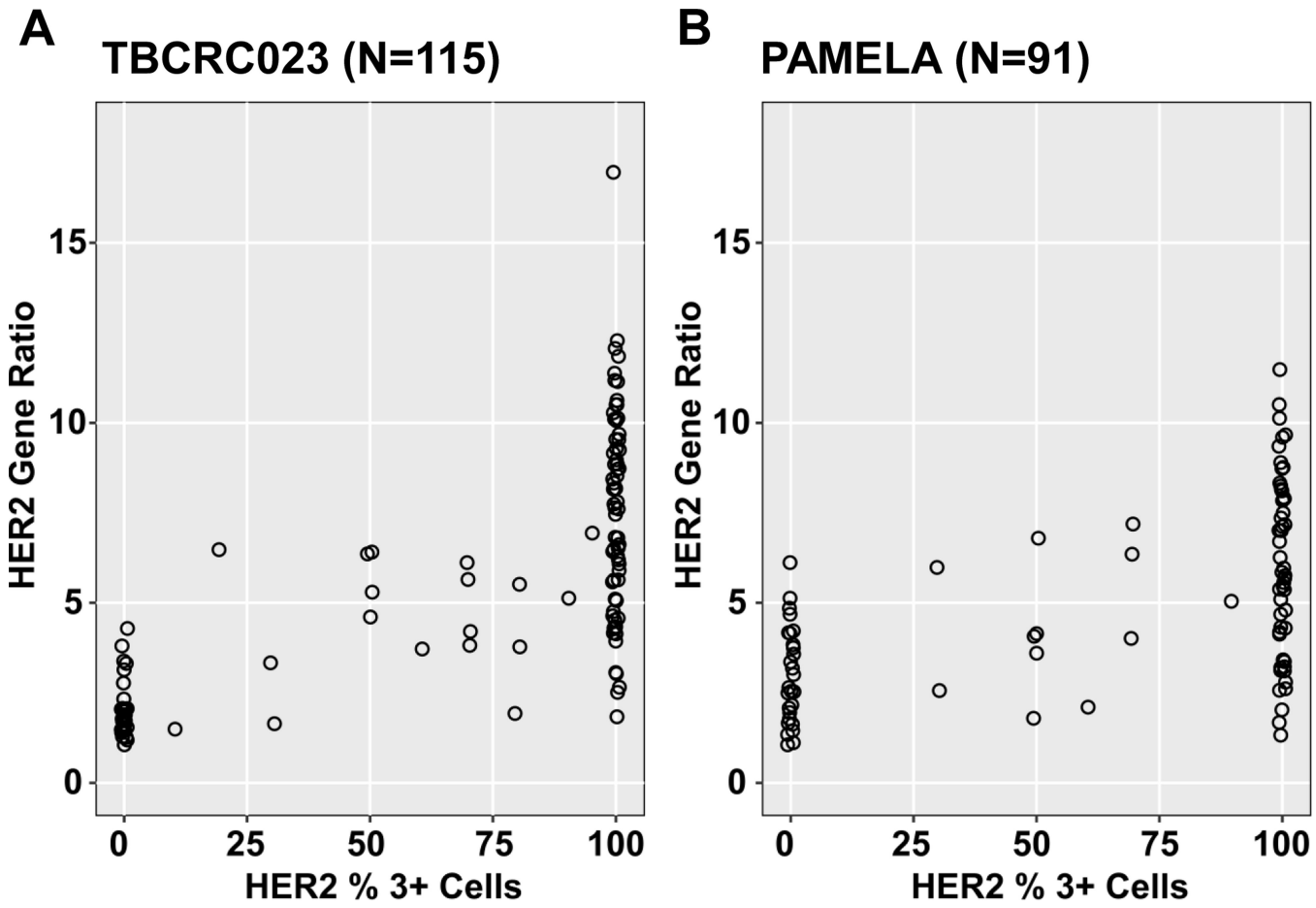

Figure 1. Distribution of HER2 gene ratio and protein in TBCRC023 and PAMELA neoadjuvant cohorts.

Dot plots showing the distribution of HER2 gene ratio and percentage of HER2 3+ protein staining measured using the dual Gene Protein Assay in evaluable baseline specimens of the (**A**) TBCRC023 and (**B**) PAMELA cohort. Each circle represents an individual specimen.

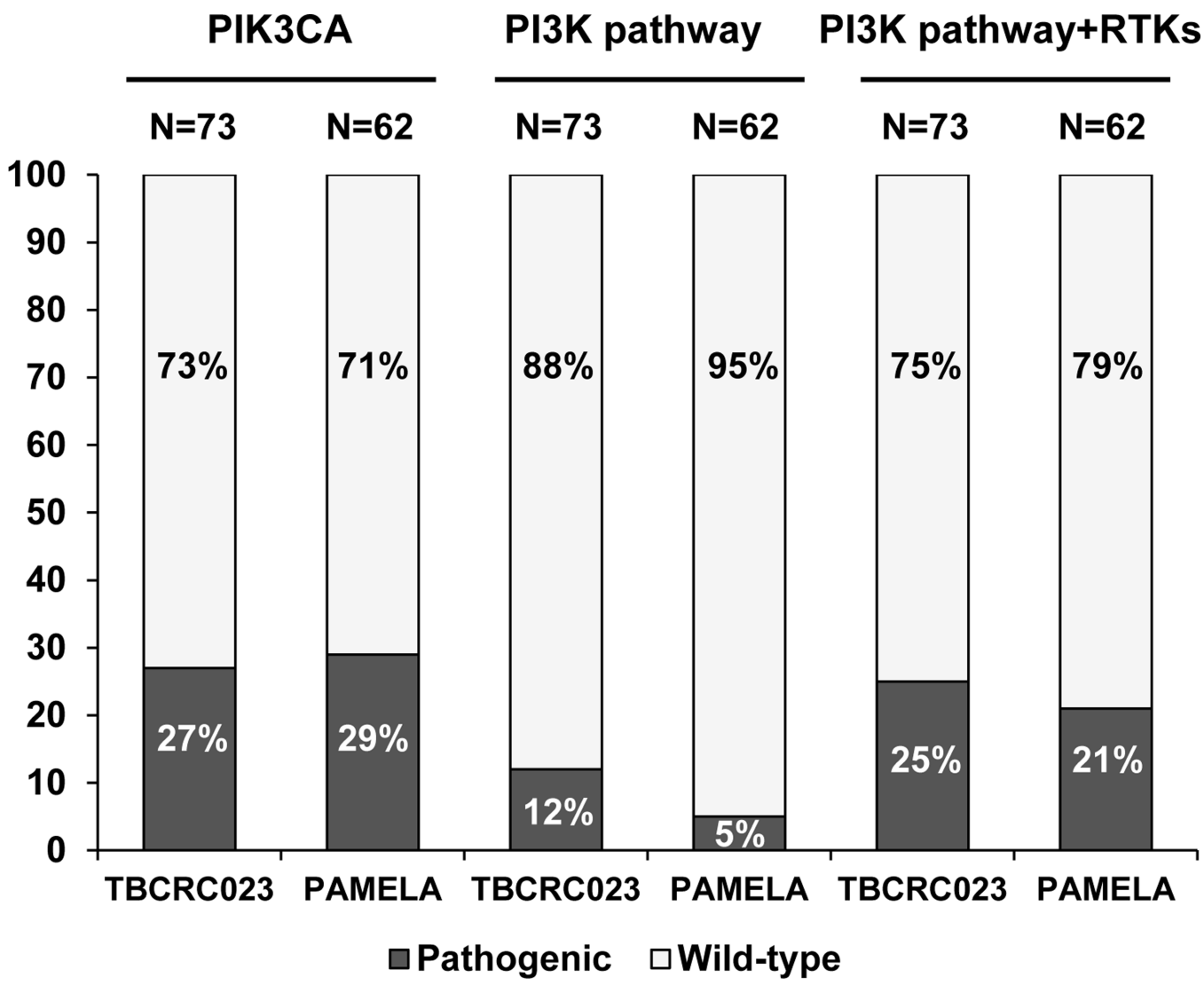

Figure 2. Pathogenic genetic alterations in TBCRC023 and PAMELA specimens.

Stacked bar graphs showing the percentage of pathogenic genetic alterations detected in the *PIK3CA* gene, PI3K pathway, or PI3K pathway+receptor tyrosine kinases (RTKs) in the evaluable specimens of the TBCRC023 and PAMELA cohorts.

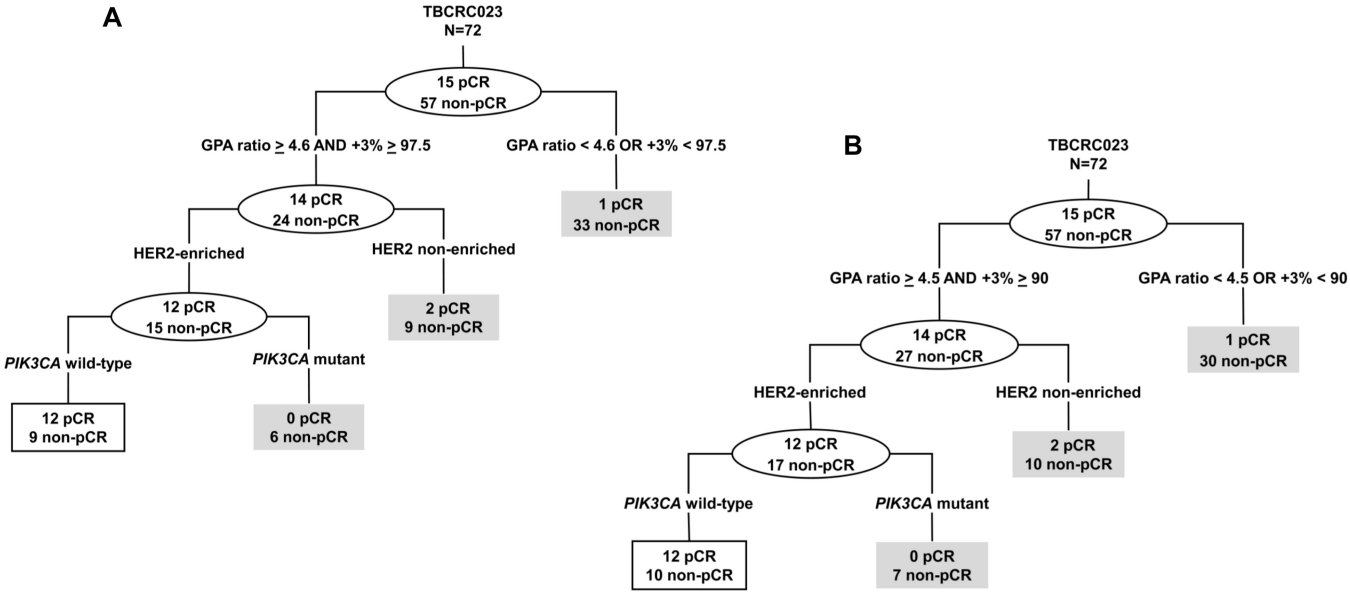

Figure 3. Decision tree algorithm to construct the multiparameter classifier of response.

Decision tree algorithms constructed using evaluable specimens in the TBCRC023 cohort with data for all three biomarkers show the components that make up the (**A**) empirical molecular classifier and (**B**) molecular classifier. The top tier represents the trunk of the tree with the total number of evaluable specimens and a breakdown of the pCR and non-pCR cases. The branches lead to decision nodes that contain a splitting attribute. Ovals on the left reflect cases that fit the respective splitting attribute and are therefore passed on to subsequent nodes for further selection. The white rectangle at the bottom left defines the terminal node of the tree and the final criteria of the classifier. Gray rectangles on the right reflect cases that did not meet the splitting criteria and therefore fell off the tree.

Table 1.

Patient characteristics of the neoadjuvant cohorts at baseline.

	All evaluable (A)				Complete cohort (B)			*P*-value (B vs A)		Partial cohort (C)		
	Overall	**TBCRC023**	**PAMELA**	***P*-value**	**Overall**	**TBCRC023**	**PAMELA**	**TBCRC023**	**PAMELA**	**Overall**	**TBCRC023**	**PAMELA**
No.	273	124	149		116	72	44			93	29	64
Age, y, Mean (SD)	54.1 (12.4)	53.1 (11.8)	54.9 (12.9)	0.22	53.3 (11.1)	53.3 (11.4)	53.4 (10.9)	0.866	0.322	54.9 (13.5)	53.9 (13.6)	55.4 (13.6)
Menopausal status (%)				0.044				0.059	0.936			
Premenopausal	126 (46.2)	66 (53.2)	60 (40.3)		61 (52.6)	44 (61.1)	17 (38.6)			39 (41.9)	12 (41.4)	27 (42.2)
Postmenopausal	147 (53.8)	58 (46.8)	89 (59.7)		55 (47.4)	28 (38.9)	27 (61.4)			54 (58.1)	17 (58.6)	37 (57.8)
Tumor stage (%)				<0.001				0.288	0.726			
T1	64 (23.6)	4 (3.3)	60 (40.3)		19 (16.4)	1 (1.4)	18 (40.9)			28 (30.4)	2 (7.1)	26 (40.6)
T2	149 (55.0)	70 (57.4)	79 (53.0)		66 (56.9)	44 (61.1)	22 (50.0)			49 (53.3)	15 (53.6)	34 (53.1)
T3–4	58 (21.4)	48 (39.3)	10 (6.7)		31 (26.7)	27 (37.5)	4 (9.1)			15 (16.3)	11 (39.3)	4 (6.2)
Nodal status (%)				0.866				0.489	0.832			
N	179 (66.5)	81 (67.5)	98 (65.8)		75 (65.8)	45 (64.3)	30 (68.2)			63 (68.5)	23 (82.1)	40 (62.5)
Y	90 (33.5)	39 (32.5)	51 (34.2)		39 (34.2)	25 (35.7)	14 (31.8)			29 (31.5)	5 (17.9)	24 (37.5)
Treatment duration (%)				<0.001				0.558	NA			
12 Weeks	43 (15.8)	43 (34.7)	0 (0.0)		27 (23.3)	27 (37.5)	0 (0.0)			12 (12.9)	12 (41.4)	0 (0.0)
18 Weeks	149 (54.6)	0 (0.0)	149 (100.0)		44 (37.9)	0 (0.0)	44 (100.0)			64 (68.8)	0 (0.0)	64 (100.0)
24 Weeks	81 (29.7)	81 (65.3)	0 (0.0)		45 (38.8)	45 (62.5)	0 (0.0)			17 (18.3)	17 (58.6)	0 (0.0)
Hormone receptor (%)				0.005				0.62	1			
HR+	159 (58.2)	84 (67.7)	75 (50.3)		69 (59.5)	47 (65.3)	22 (50.0)			60 (64.5)	19 (65.5)	41 (64.1)
HR-	114 (41.8)	40 (32.3)	74 (49.7)		47 (40.5)	25 (34.7)	22 (50.0)			33 (35.5)	10 (34.5)	23 (35.9)
Outcome (%)				0.042				0.795	0.974			
pCR	70 (25.6)	24 (19.4)	46 (30.9)		28 (24.1)	15 (20.8)	13 (29.5)			14 (15.1)	2 (6.9)	12 (18.8)
Non-pCR	203 (74.4)	100 (80.6)	103 (69.1)		88 (75.9)	57 (79.2)	31 (70.5)			79 (84.9)	27 (93.1)	52 (81.2)

y, years; SD, standard deviation; N, No; Y, Yes; HR+, hormone receptor positive; HR-, hormone receptor negative; pCR, pathologic complete response.

Table 2.

Performance of the empirical molecular classifier and molecular classifier in complete cohorts.

Empirical molecular classifier	**pCR**	**non-pCR**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
TBCRC023 (*N*=72)			80%	84%	57%	94%
Predict pCR	12	9				
Predict non-pCR	3	48				
PAMELA (*N*=44)			62%	74%	50%	82%
Predict pCR	8	8				
Predict non-pCR	5	23				
Molecular classifier	**pCR**	**non-pCR**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
TBCRC023 (*N*=72)			80%	83%	55%	94%
Predict pCR	12	10				
Predict non-pCR	3	47				
PAMELA (*N*=44)			62%	71%	47%	82%
Predict pCR	8	9				
Predict non-pCR	5	22				

pCR, pathologic complete response; PPV, positive predictive value; NPV, negative predictive value.

Table 3.

Performance of the molecular classifier in complete and partial cohorts with reassessed GPA data.

Complete cohort	**pCR**	**non-pCR**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
TBCRC023 (*N*=72)			73	83	52	92
Predict pCR	11	10				
Predict non-pCR	4	47				
PAMELA (*N*=45)			64	77	56	83
Predict pCR	9	7				
Predict non-pCR	5	24				
Partial cohort	**pCR**	**non-pCR**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
TBCRC023 (*N*=31)						94
Predict non-pCR	2	29				
PAMELA (*N*=63)						83
Predict non-pCR	11	52				

pCR, pathologic complete response; PPV, positive predictive value; NPV, negative predictive value.