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Annals of Oncology 30: 927–933, 2019
doi:10.1093/annonc/mdz076
Published online 23 March 2019

ORIGINAL ARTICLE

A combinatorial biomarker predicts pathologic complete response to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2+ breast cancer

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Background: HER2-positive (+) breast cancers, defined by HER2 overexpression and/or amplification, are often addicted to HER2 to maintain their malignant phenotype. Yet, some HER2+ tumors do not benefit from anti-HER2 therapy. We hypothesize that *HER2* amplification levels and PI3K pathway activation are key determinants of response to HER2-targeted treatments without chemotherapy.

Patients and methods: Baseline HER2+ tumors from patients treated with neoadjuvant lapatinib plus trastuzumab [with endocrine therapy for estrogen receptor (ER)+ tumors] in TBCRC006 (NCT00548184) were evaluated in a central laboratory for *HER2* amplification by fluorescence *in situ* hybridization (FISH) ($n = 56$). *HER2* copy number (CN) and FISH ratios, and PI3K pathway status, defined by *PIK3CA* mutations or PTEN levels by immunohistochemistry were available for 41 tumors. Results were correlated with pathologic complete response (pCR; no residual invasive tumor in breast).

Results: Thirteen of the 56 patients (23%) achieved pCR. None of the 11 patients with *HER2* ratio <4 and/or CN <10 achieved pCR, whereas 13/45 patients (29%) with *HER2* ratio ≥ 4 and/or CN ≥ 10 attained pCR ($P = 0.0513$). Of the 18 patients with tumors expressing high PTEN or wild-type (WT) *PIK3CA* (intact PI3K pathway), 7 (39%) achieved pCR, compared with 1/23 (4%) with PI3K pathway alterations ($P = 0.0133$). Seven of the 16 patients (44%) with *HER2* ratio ≥ 4 and intact PI3K pathway achieved pCR, whereas only 1/25 (4%) patients not meeting these criteria achieved pCR ($P = 0.0031$).

Conclusions: Our findings suggest that there is a clinical subtype in breast cancer with high *HER2* amplification and intact PI3K pathway that is especially sensitive to HER2-targeted therapies without chemotherapy. A combination of *HER2* FISH ratio and PI3K pathway status warrants validation to identify patients who may be treated with HER2-targeted therapy without chemotherapy.

Key words: ErbB2 receptor tyrosine kinase, breast cancer, fluorescent *in situ* hybridization, *PIK3CA* mutations, PTEN protein, precision medicine

Introduction

HER2-positive (+) breast cancer (BC), an aggressive subtype accounting for 15%–20% of BCs, is defined by *HER2* (*ERBB2*) gene amplification and/or protein overexpression. Since its FDA approval for treatment of HER2+ BC with combination chemotherapy, trastuzumab (T) has markedly improved patient outcome. We and others have shown that multi-agent anti-HER2 therapy is superior to single agents in achieving comprehensive HER receptor family blockade and delivering potent antitumor effects.

In our preclinical studies, multi-agent anti-HER2 treatment, combined with endocrine therapy in estrogen receptor-positive (ER)+ tumor models, but without chemotherapy yielded complete tumor eradication [1–3]. We therefore posited that for a subset of HER2-amplified BCs, targeted therapy alone would suffice and that the inclusion of chemotherapy may not offer meaningful benefit. This treatment de-escalation approach was the central hypothesis investigated in the neoadjuvant TBCRC006 trial, in which patients with HER2+ BC were treated with 12 weeks of lapatinib (L) +T (without chemotherapy), plus endocrine therapy if the tumor was ER+ [4]. In this trial, 27% of the patients achieved pathologic complete response (pCR) despite a median tumor size of 6 cm. This clinically meaningful pCR rate has now been confirmed in two similar neoadjuvant trials TBCRC023 and PAMELA, which stress the need to differentiate patients appropriate for this de-escalation strategy from those needing additional therapy [5, 6].

Germane to the success of future de-escalation trials for HER2+ BC patients is to identify patients whose tumors are truly 'addicted to' (i.e. physiologically dependent on) HER2 signaling and, therefore, may benefit from HER2-targeted therapy alone. The current ASCO/CAP guidelines define HER2 positivity as fluorescence *in situ* hybridization (FISH) ratio $\geq$2, *HER2* gene copy number (CN) $\geq$6.0, or immunohistochemistry (IHC) score 3+ [7, 8]. These cut-offs were largely defined in patients receiving T plus chemotherapy, which may not be optimal for a chemotherapy-sparing treatment regimen where true reliance on HER2 signaling (i.e. oncogene addiction) would be required for response.

Whilst *HER2* gene amplification is the yardstick for anti-HER2 therapy, some tumors do not benefit from anti-HER2 therapy despite harboring high HER2 levels. Preclinical studies have suggested that *PIK3CA* mutations and/or PTEN loss of expression are associated with or causative of resistance to anti-HER2 therapies. Our recent molecular analysis of TBCRC006 HER2+ BCs has provided clinical evidence supporting these findings, given that activation of the PI3K pathway by either *PIK3CA* activating mutations or low levels of the tumor suppressor PTEN were associated with resistance to L + T, plus endocrine therapy, in the absence of chemotherapy [9].

We hypothesized that low levels of *HER2* amplification might be insufficient to cause HER2 addiction and that PI3K pathway deregulation would activate the pathway downstream of HER2, thereby causing resistance to therapies targeting HER2 and other HER family members at the cell membrane. In support of this hypothesis, we carried out an exploratory analysis of *HER2* gene amplification, ER status and other markers along the HER2/ER signaling axis, and deregulation of the PI3K pathway in baseline biopsies from the previously reported TBCRC006 trial and their association with pCR after 12 weeks of therapy.

Methods

Patients and clinical specimens

Study design, patient characteristics (median tumor size 6 cm), and patient outcome [measured by pCR (no residual invasive tumor in the breast; ypT0-is), and near pCR (npCR; residual invasive disease of $\leq$1 cm (ypT1a-b) at time of surgery)] in neoadjuvant TBCRC006 (NCT00548184) trial have been previously reported [4]. In this correlative study, baseline core biopsies were evaluated for various biomarkers (supplementary Figure S1A, available at *Annals of Oncology* online).

Immunohistochemistry

IHC was carried out using the antibodies described in supplementary Table S1, available at *Annals of Oncology* online. ER, progesterone receptor (PR) and HER2 were carried out according to the ASCO/CAP Guidelines [7, 8, 10] (supplementary Methods, available at *Annals of Oncology* online).

PI3K pathway alterations

Alterations affecting canonical components of the PI3K pathway (i.e. *PIK3CA* mutations or low PTEN expression, defined as *H*-score <median of 100) were available for tumors from 43 assessable patients from our recently published TBCRC006 molecular study [9].

HER2 FISH assay

FISH assay was carried out using the PathVysion *HER2* DNA Probe kit (Abbott Molecular, Abbott Park, IL) per the manufacturer's instructions. Determination of *HER2*/neu gene amplification was achieved by calculating the ratio of average *HER2*/*neu* probe signals to the average CEP17 probe signals. The *HER2* FISH ratios and average *HER2* gene CN were calculated and interpreted according to standard guidelines by two pathologists blinded to the results of the molecular analyses and response to therapy.

Statistical analysis

Biomarker data were summarized descriptively. Spearman's rank correlation was used to assess correlations among the biomarkers. Associations between biomarkers and pathological response were examined with Fisher's exact tests or Wilcoxon rank-sum tests, as appropriate. Odds ratios and 95% conference intervals were calculated to measure strength of associations. *P*-values <0.05 were considered statistically significant. In view of the exploratory nature of this analysis, there is no adjustment for multiple comparisons. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Of the 64 assessable patients, 17 (27%) attained pCR following L + T treatment (with endocrine therapy for ER+ tumors) [4, 9]. Evaluable baseline tumor specimens were analyzed for individual biomarkers based on tissue availability (supplementary Figure S1B, available at *Annals of Oncology* online).

Expression and correlation of baseline biomarkers

A significant positive correlation was observed in the expression of ER with two of its key downstream gene products, PR and BCL2. Conversely, a significant inverse correlation was observed between HER2 expression measured by IHC, and ER, PR and BCL2, as well as between ER and pMAPK. Statistically significant positive correlations between *HER2* FISH ratio, CN and *H*-score were also observed (supplementary Table S2, available at *Annals of Oncology* online). No significant differences, however, were observed overall in the expression levels of biomarkers between the pCR versus no pCR groups (supplementary Table S3, available at *Annals of Oncology* online).

HER2 gene amplification/CN and anti-HER2 treatment response

The mean and median of *HER2* FISH ratio were 6.34 and 5.75, respectively, whereas the mean and median *HER2* CN were 14.13 and 13.65, respectively. The mean and median HER2 protein *H*-scores were 245.41 and 300, respectively (supplementary Table S4, available at *Annals of Oncology* online). No significant differences in the median *HER2* FISH ratio ($P = 0.3416$), CN ($P = 0.1402$) and *H*-scores ($P = 0.2336$) were observed between the pCR versus no pCR groups (supplementary Table S3, available at *Annals of Oncology* online).

Of the 56 patients, 13 (23%) achieved pCR [9] (Figure 1A; Table 1). None of the patients with an *HER2* FISH ratio <4 and/or CN <10 (green dashed lines, Figure 1A) achieved pCR, whereas 13/45 patients (29%) with tumors above this threshold (*HER2* FISH ratio ≥4 and/or CN ≥10) attained pCR ($P = 0.0513$). Nevertheless, 71% (32/45) of the patients with tumors above this threshold did not achieve pCR, despite harboring high *HER2* ratios and CN levels, suggesting that other factors may also contribute to treatment resistance.

Impact of combined *HER2* gene amplification and PI3K pathway status

The analysis of baseline samples from TBCRC006 has recently demonstrated that PI3K pathway deregulation by *PIK3CA* mutation or low PTEN expression (*H*-score <median of 100), is associated with resistance to anti-HER2 treatments [9]. Indeed, 15/23 (65%) HER2-overexpressing tumors that did not achieve pCR harbored *PIK3CA* mutations or low PTEN levels (NR group, Figure 1B), suggesting that PI3K pathway activation by alterations downstream of HER2 may explain the majority of *HER2*-amplified cases that are resistant to anti-HER2 therapies in the absence of chemotherapy.

Baseline biopsies from 41 patients were available for the evaluation of *HER2* FISH ratios and PI3K pathway alterations. The prevalence of high *HER2* CN level was numerically superior, albeit statistically non-significant, in the HER2+ BC patients that evolved to pCR, with 8/35 (23%) patients with FISH ratio ≥4 and/or CN ≥10 ($P = 0.3229$) attaining pCR, whereas none of the 6 patients with a ratio <4 or CN <10 attained pCR (Table 1). Of the 18 patients whose tumors expressed high PTEN or lacked *PIK3CA* mutations (intact PI3K pathway), 7 patients (39%) achieved pCR, as opposed to pCR in only 1/23 (4%) patients with tumors harboring PI3K pathway deregulations ($P = 0.0133$).

Combined analysis of *HER2* FISH ratio and PI3K pathway status demonstrated that 7 of the 16 patients (44%) with *HER2* FISH ≥4, and wild-type (WT) *PIK3CA* or high PTEN achieved pCR, compared with pCR in only 1 of the 25 (4%) patients whose tumors had a low *HER2* FISH ratio OR PI3K pathway alteration ($P = 0.0031$; Table 1). Likewise, 7 of the 15 (47%) patients with tumors with CN ≥10, and WT *PIK3CA* or high PTEN achieved pCR, compared with only 1/26 (5%) patients whose tumors had lower *HER2* OR a PI3K pathway alteration ($P = 0.0018$).

An exploratory analysis of treatment end point by ER status

Previous studies suggest that maximal treatment response in patients with ER+ tumors may require treatment durations longer that 12 weeks. Preliminary reports from a recent trial comparing 12 versus 24 weeks using the same regimen as TBCRC006 showed that pCR rate was higher with longer treatment duration in this subset [6]. In the present study, 77% (10/13) of patients in the npCR group had ER+ tumors, as defined by central IHC. Hence, we posited that many of the patients with ER+ tumors that did not evolve to pCR after 12 weeks might achieve pCR with longer therapy. We therefore carried out an exploratory analysis by combining pCR plus npCR to measure 'modified response' for ER+ tumors (Table 2). Eighty-one percent (13/16) of patients with tumors displaying *HER2* FISH ≥4, WT *PIK3CA* and high PTEN responded to anti-HER2 therapy as opposed to only 16% (4/25) of the patients with tumors not meeting these criteria ($P < 0.0001$). Likewise, 80% (12/15) of the patients having tumors with *HER2* CN ≥10, WT *PIK3CA* or high PTEN responded to anti-HER2 therapy compared with only 19% (5/26) in patients not meeting this criteria ($P = 0.0002$).

Discussion

The prevailing therapeutic approach for clinical management of HER2+ BC is the addition of biologic HER2-targeted treatments to chemotherapy. The mounting toxicity and cost associated with potentially unnecessary inclusion of chemotherapy should also be considered. A balanced approach to reduce overtreatment, without compromising patient outcome would constitute a paradigm shift in the treatment of HER2+ BC.

The neoadjuvant TBCRC006 was a pioneering study in that (i) chemotherapy was omitted from the 12-week L + T regimen, while endocrine therapy was given for ER+ disease [4], and (ii) results from biomarker analysis of chemotherapy-naïve tumors are slated to guide the development of a molecular approach for treatment de-escalation. Twenty-seven of the patients achieved pCR with HER2-targeted therapy alone. These findings have set the stage for subsequent chemotherapy-sparing neoadjuvant trials TBCRC023, which assessed the impact of extended anti-HER2 treatment (12 versus 24 weeks of same regimen as TBCRC006) [6], and PAMELA, which tested an identical dual inhibition regimen but for 18 weeks. Outcomes from these three trials together (>300 patients) revealed that anti-HER2 therapy alone may be beneficial for 20%–30% of HER2+ patients. Germane to the clinical success of this strategy, however, is accurate patient selection,

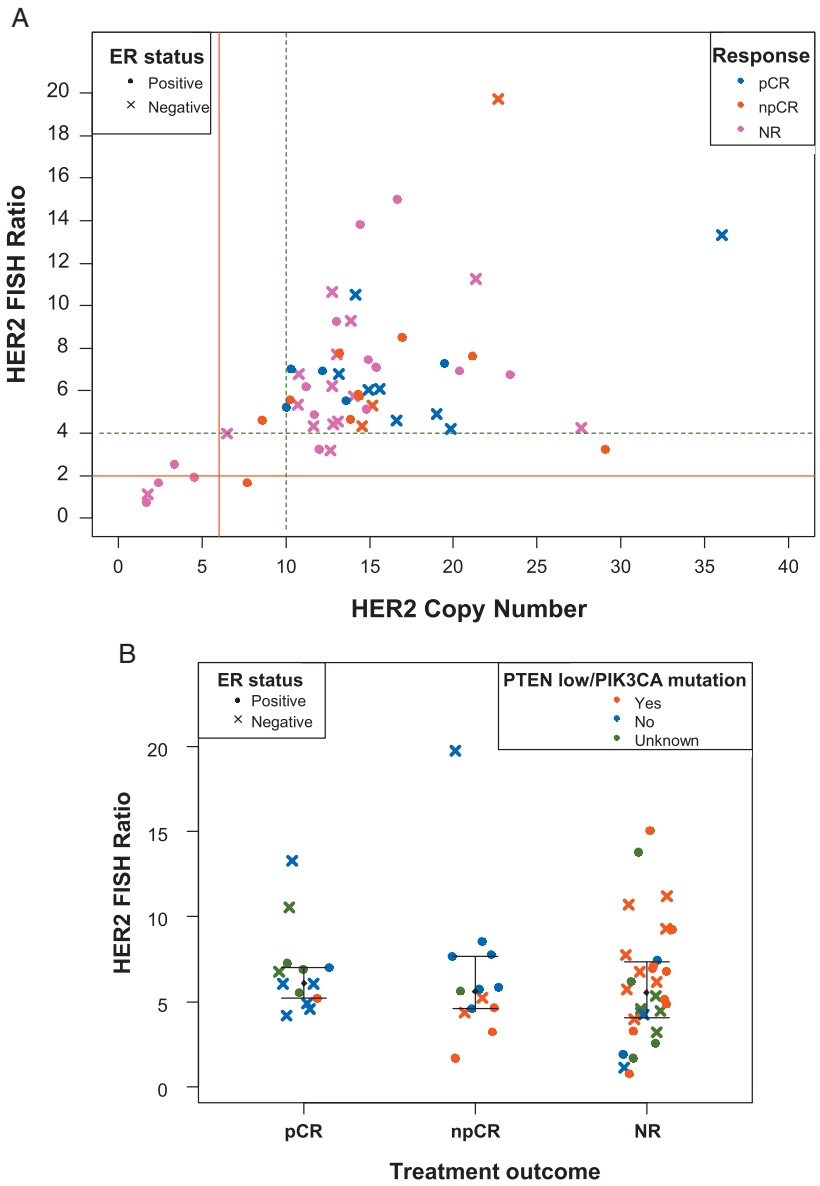

Figure 1. (A) Correlation between *HER2* copy number (CN) and gene expression ratio in baseline HER2+ tumors. Scatter plot showing the correlation between *HER2* CN and gene expression ratio in 56 evaluable baseline specimens examined by FISH with each dot representing a specimen. Solid red lines denote the ASCO cut-off guidelines for HER2+ tumors. Dashed green lines represent the threshold from our analysis. (B) Distribution of *HER2* gene expression ratio in baseline HER2+ tumors. Dot plot showing the distribution of *HER2* gene expression ratio in relation to treatment outcome [pCR, near pCR (npCR), and non-responder (NR)] with each dot representing a specimen.

which requires the development of biomarkers with adequate positive and negative predictive values.

The effectiveness of HER2-targeted therapy is determined by a multitude of factors. Our study provides evidence to suggest that principal among these factors are the levels of *HER2* gene amplification, which may constitute a surrogate of HER2 'oncogene addiction' and the absence of known mechanisms of activation of the PI3K pathway. Our exploratory combinatorial analysis of biomarkers identifies a possible clinical subtype that is bona fide HER2-addicted and exhibits exquisite sensitivity to anti-HER2 therapy in the absence of chemotherapy. This, however, does not preclude the benefit of adding anti-HER2 agents to chemotherapy in other subsets of HER2+ tumors that may not be as addicted to HER2. In the neoadjuvant setting, *ERBB2* mRNA expression was highly associated with pCR in CALGB40601 [11] and NeoALTTO [12]. In contrast, the HERA trial reported that the degree of *HER2* amplification did not influence response to adjuvant trastuzumab with chemotherapy [13], a finding also observed in the N9831 adjuvant trastuzumab trial [14]. This is not surprising given that, unlike our study where patients solely received anti-HER2 therapy (and endocrine therapy in patients with ER+ disease), patients included in those studies [11–13] received a combination of anti-HER2 therapies and chemotherapy. The unique cohort of chemotherapy-naïve patients with HER2+ tumors allowed us to explore this uncharted territory, and confirmed previous preclinical observations supportive of

Table 1. *HER2* FISH ratio, copy number, and PI3K pathway deregulations, determined by low PTEN expression levels and *PIK3CA* mutations, in baseline tumor samples as predictive markers of pCR response to the trastuzumab + lapatinib regimen

Marker	All	pCR		npCR/NR		*P*-value*	OR	95% CI
	n	*n*	%	*n*	%			
***HER2* FISH ratio (*n* =41)**						0.3229		
≥4	35	8	23	27	77		4.02	0.163–98.988
<4	6	0	–	6	100		1	–
***HER2* copy number (*n*=41)**						0.3229		
≥10	35	8	23	27	77		4.02	0.163–98.988
<10	6	0	–	6	100		1	–
PTEN low or *PIK3CA* mutation (*n* = 41)						0.0133		
No (intact PI3K pathway)	18	7	39	11	61		13.999	1.525–128.467
Yes (deregulated PI3K pathway)	23	1	4	22	96		1	–
***HER2* FISH ratio/PTEN low or *PIK3CA* mutation (*n* =41)**						0.0031		
HER2 FISH ratio ≥4, intact PI3K pathway (PTEN low or *PIK3CA* mutation=no)	16	7	44	9	56		18.664	2.005–173.711
Others	25	1	4	24	96		1	–
***HER2* copy number/PTEN low or *PIK3CA* mutation (*n*=41)**						0.0018		
HER2 copy number ≥10, intact PI3K pathway (PTEN low or *PIK3CA* mutation=no)	15	7	47	8	53		21.87	2.325–205.696
Others	26	1	4	25	96		1	–

*P-values were calculated by Fisher's exact test.
HER2, human epidermal growth factor receptor 2; PI3K, phosphoinositol-3 kinase; pCR, pathologic complete response; npCR, near pCR; NR, non-responder; OR, odds ratio; PTEN, phosphatase and tensin homolog; CI, confidence interval.

Table 2. *HER2* FISH ratio, copy number, and PI3K pathway deregulations, determined by low PTEN expression levels and *PIK3CA* mutations, in baseline tumor samples as predictive markers of Modified response to the trastuzumab + lapatinib regimen

Marker	All	Modified response (ER+=pCR+npCR; ER−=pCR)		No response (ER+=NR; ER−=npCR+NR)		*P*-value*	OR	95% CI
	n	*n*	%	*n*	%			
***HER2* FISH ratio (*n*=41)**						1		
≥4	35	15	43	20	57		1.5	0.242–9.3
<4	6	2	33	4	67		1	–
***HER2* copy number (*n*=41)**						1		
≥10	35	15	43	20	57		1.5	0.242–9.3
<10	6	2	33	4	67		1	–
PTEN low or *PIK3CA* mutation (*n*=41)						0.0011		
No (intact PI3K pathway)	18	13	72	5	28		12.349	2.777–54.908
Yes (deregulated PI3K pathway)	23	4	17	19	83		1	–
***HER2* FISH ratio/PTEN low or *PIK3CA* mutation (*n*=41)**						<0.0001		
HER2 FISH ratio ≥4, intact PI3K pathway (PTEN low or *PIK3CA* mutation=no)	16	13	81	3	19		22.746	4.373–118.318
Others	25	4	16	21	84		1	–
***HER2* copy number/PTEN low or *PIK3CA* mutation (*n*=41)**						0.0002		
HER2 copy number ≥10, intact PI3K pathway (PTEN low or *PIK3CA* mutation=no)	15	12	80	3	20		16.799	3.4–82.995
Others	26	5	19	21	81			–

**P*-values were calculated by Fisher's exact test.
HER2, human epidermal growth factor receptor 2; PI3K, phosphoinositol-3 kinase; pCR, pathologic complete response; npCR, near pCR; NR, non-responder; OR, odds ratio; PTEN, phosphatase and tensin homolog; CI, confidence interval.

the notion that *PIK3CA* mutations and loss of PTEN expression can result in resistance to anti-HER2 agents [15, 16].

Whilst the ASCO/CAP guidelines [7] for adding trastuzumab to chemotherapy identifies tumors with FISH ratio ≥2.0, or CN ≥6.0 as HER2+, in our cohort, none of the patients with FISH ratio <4 and/or CN <10 tumor achieved pCR. About 27% of the patients with FISH ratio ≥4 and CN ≥10 attained pCR despite the large tumor size. This supports the contention that for patients with bona fide HER2-addicted tumors (i.e. that may be sensitive to anti-HER2 therapy alone), the threshold for HER2 may need to be set higher than that proposed in the current guidelines. Although validation of such a threshold in a larger patient cohort is needed, our findings would support the notion that the HER2-addicted subtype of HER2+ BCs might be identifiable on the basis of *HER2* FISH combined with additional molecular assays (e.g. PAM50 subtyping and PI3K pathway alterations). Consistent with this notion, the PAMELA trial demonstrated that in the absence of chemotherapy, the response to anti-HER2 therapies in patients with HER2+ BC varied according to intrinsic subtype, with a 41% pCR rate in the HER2-enriched (HER2-E) subtype and only 10% in the non-HER2-E subtype. Yet, a large proportion of patients whose tumors displayed an *HER2* FISH ratio ≥4 and/or CN ≥10 or an HER2-E subtype failed to achieve pCR. Our findings here suggest that this phenomenon could be explained in part by PI3K pathway alterations. We have recently reported that deregulation of the PI3K pathway, as defined by *PIK3CA* mutations or low PTEN expression, is associated with significantly lower response to anti-HER2 therapy in the TBCRC006 trial. Anti-HER2 therapy in combination with chemotherapy yielded inferior response rates in patients with *PIK3CA* mutations in both neoadjuvant [17, 18] and adjuvant [19] settings. The clinical association of PTEN levels with pCR, on the other hand, is conflicting (reviewed in [20]). One probable explanation for this discordance is the administration of chemotherapy, which could have confounded the association and masked resistance to pure anti-HER2 therapy since tumors with *PIK3CA* mutations and possibly PTEN downregulation are sensitive to chemotherapy. In this study, we document the potential of HER2 levels and PI3K pathway status together in predicting response to HER2-targeted therapy.

Preclinical evidence demonstrate that ER should be blocked together with HER2 for complete tumor eradication. In the chemotherapy-sparing TBCRC023 study, we demonstrated that longer treatment duration resulted in increased pCR (from 12% at 12 weeks to 28% at 24 weeks), probably by converting the npCR cases to pCR, especially in the ER+ group (9% versus 33% in 12 versus 24 weeks) [6]. It is plausible that some of the npCR cases that we reported in the TBCRC006 study would have achieved pCR with longer treatment. To test this, we carried out a 'modified response' analysis by considering npCR plus pCR as 'modified response' for patients with ER+ tumors, which showed that 81% of the patients with *HER2* FISH ≥4, WT *PIK3CA* and high PTEN tumors achieved response, as opposed to only 16% response in patients with tumors not meeting the criteria. Although there is no direct evidence supporting npCR as a prognostic marker of response specifically in ER+/HER2+ tumors, the findings from our exploratory analysis support the argument that tumors with active ER signaling may benefit from longer treatment durations.

This study has several limitations. Aside from the relatively small patient cohort, we were also limited by tissue availability for collective analysis of *HER2* FISH, *PIK3CA* mutations and PTEN expression. The small sample size could explain the failure to achieve statistical significance for difference in pCR between tumors with *HER2* FISH ≥4 and <4, which however, was achieved when *HER2* FISH was combined with *PIK3CA* mutation status and PTEN expression. In addition, owing to limitations in tissue sample availability, we have only investigated alterations affecting *PIK3CA* and PTEN, the two most frequently altered components of the canonical PI3K pathway. It is entirely plausible that the prediction of pCR would have been more precise if additional components of the PI3K pathway had been analyzed.

Despite these limitations, our study supports the potential of combining easily assessable biomarkers from two key pathways to identify HER2+ patients who may benefit from anti-HER2 therapies without chemotherapy. Additional validation of these results in larger and independent patient cohorts is warranted. Overall, our results accentuate the need for tailored therapy to improve the quality of life through treatment de-escalation. These hypothesis generating findings may inspire a new generation of studies to develop molecular predictors to effectively triage patients for appropriate and beneficial treatments.

Acknowledgements

We thank the Specialized Program of Research Excellence (SPORE) Pathology Core laboratory at the Lester and Sue Smith breast center in Baylor College of Medicine for performing the *HER2* FISH assay.

Funding

This work was supported in part by the NIH: SPORE Grants (P50 CA058183 and CA186784 to RS, CKO and MFR); Cancer Center Grants (P30 CA125123, P30 CA008748); the Breast Cancer Research Foundation (to RS, CKO and JSR-F; no grant number applies); research grant from the Breast Cancer Research Foundation BCRF-17-143 (to RS and CKO); the Cancer Prevention & Research Institute of Texas CPRIT RP 140102 and the Conquer Cancer Foundation—Gianni Bonadonna Breast Cancer Research Fellowship (to CDA); Department of Defense grants W81XWH-17-1-0579 (to MFR) and W81XWH-17-1-0580 (to RS), and the Translational Breast Cancer Research Consortium (TBCRC). We are grateful for the funding support to the TBCRC from The AVON Foundation, the Breast Cancer Research Foundation and Susan G. Komen.

Disclosure

AF: research funding from Genentech (to Institution). RN: research funding from Celgene, Corcept, and Merck. Advisory boards for AstraZeneca, Celgene, Genentech, Merck, Daiichi, Macrogenics, Syndax, and PUMA biotechnology. MPG: research funding from Lilly and Pfizer. Consulting/Advisory boards for Lilly, Biovica, Novartis, Sermonix, and Genomic Health. JCC: Research funding from Novartis, Celgene,

AstraZeneca, Merck, and Takeda; Advisory boards for Genentech, and Celgene. AP: research funding from Nanostring Technologies, Advisory boards for Nanostring Technologies, Novartis, and Roche. JSR-F: consultance fees from Goldman Sachs, Scientific Advisory Boards of Volition Rx and Paige.AI, Ad hoc Scientific Advisory Boards of Ventana, Genentech and Roche. CG: research grant from Ventana. CKO: research funding from AstraZeneca and GlaxoSmithKline, Advisory boards for Tolmar Pharmaceuticals, Genentech, and AstraZeneca, DMC for Eli Lilly, stockholder of GeneTex. MFR: Research funding from GlaxoSmithKline and Genentech. RS: Research funding from AstraZeneca, GlaxoSmithKline, Gilead Sciences, and PUMA Biotechnology; Consulting/advisory committee member for Macrogenics, and Eli Lilly. All remaining authors have declared no conflicts of interest.

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