Published OnlineFirst December 2, 2014; DOI: 10.1158/1078-0432.CCR-14-1824

Personalized Medicine and Imaging

Clinical Cancer Research

High HER2 Expression Correlates with Response to the Combination of Lapatinib and Trastuzumab

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Abstract

Purpose: Expression of p95HER2 has been associated with resistance to trastuzumab-based therapy in patients with metastatic breast cancer. Conversely, high levels of HER2 have been linked with increased clinical benefit from anti-HER2 therapy. In this work, we aimed to investigate whether the levels of p95HER2 and HER2 can predict response to anti-HER2 therapy in patients with breast cancer.

Experimental Design: We measured p95HER2 and HER2 by VeraTag and HERmark, respectively, in primary tumors of patients enrolled in the neoadjuvant phase III study NeoALTTO and correlated these variables with pathologic complete response (pCR) and progression-free survival (PFS) following lapatinib (L), trastuzumab (T), or the combination of both agents (L+T).

Results: A positive correlation between p95HER2 and HER2 levels was found in the 274 cases (60%) in which quantification of both markers was possible. High levels of these markers were predictive for pCR, especially in the hormone receptor (HR)–positive subset of patients. High HER2 expression was associated with increased pCR rate upon L+T irrespective of the HR status. To examine whether the levels of either p95HER2 or HER2 could predict for PFS in patients treated with lapatinib, trastuzumab or L+T, we fit to the PFS data in Cox models containing $\log_2$ (p95HER2) or $\log_2$(HER2). Both variables correlated with longer PFS.

Conclusions: Increasing HER2 protein expression correlated with increased benefit of adding lapatinib to trastuzumab. HER2 expression is a stronger predictor of pCR and PFS than p95HER2 for response to lapatinib, trastuzumab and, more significantly, L+T. *Clin Cancer Res; 21(3); 569–76.* ©*2014 AACR.*

Introduction

Approximately 20% of breast cancers exhibit HER2 amplification/overexpression resulting in an aggressive tumor phenotype and reduced survival (1–3). Two main therapeutic strategies have been developed over the last 15 years to treat HER2-positive breast cancer: mAbs binding to extracellular domains of the receptor (e.g., trastuzumab, pertuzumab, and T-DM1) and small molecules that inhibit the intracellular kinase domain (e.g., lapatinib, neratinib, and afatinib).

Trastuzumab (T), in combination with chemotherapy, has demonstrated a robust improvement in progression-free survival (PFS) in addition to overall survival in advanced disease (4, 5) as well as in the early (adjuvant) setting (6–8). Lapatinib (L), when given in combination with capecitabine, significantly improves time to progression in patients with HER2-positive breast cancer that have progressed on trastuzumab-based therapy, compared with capecitabine alone (9). Moreover, lapatinib as monotherapy and in combination with paclitaxel has clinical activity as first-line treatment in patients with HER2-positive breast cancer (10, 11). Recently, the antitumor activity of dual HER2 blockade (trastuzumab in combination with either pertuzumab or lapatinib) was tested in large cohorts of patients and was proven to be significantly superior to single agents (12–14).

Among "HER2-positive" tumors (defined by consensus criteria; ref. 15), there is a wide range of variability in terms of *HER2* gene amplification and protein expression measured by the conventional semiquantitative methods like the HercepTest. The possibility that a quantitative analysis of HER2 protein expression could improve prediction of sensitivity to anti-HER2 agents has led to the evaluation of alternative HER2 tests. For example, the

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-14-1824

Published OnlineFirst December 2, 2014; DOI: 10.1158/1078-0432.CCR-14-1824

Translational Relevance

The range of expression of HER2 in tumors from patients with HER2-positive breast cancer is extremely variable. In this article, we asked whether tumors with high expression of this receptor are more susceptible to respond to neoadjuvant anti-HER2 therapy. We found that patients with relatively high expression of HER2 have a significantly higher probability to achieve benefit from the combination of trastuzumab and lapatinib, two FDA-approved anti-HER2 agents currently used in the clinic. This translated in prolonged progression-free survival following the therapy. These findings indicate that HER2 quantification can be used to stratify patients that are more likely to respond to this combination. Moreover, we speculate that patients with high HER2 levels may be exquisitely sensitive to anti-HER2 therapy even in the absence of concomitant chemotherapy.

HERmark assay has been shown to provide continuous, rather than semiquantitative, measurement of HER2 proteins and their respective homodimers over a wide range of expression levels (16). Using this methodology in a retrospective study with 102 women who had received trastuzumab-based therapy, high HER2 protein expression was found to be significantly associated with better outcomes (17). Conversely, patients with tumors that expressed lower levels of HER2 received significantly lower clinical benefit from therapy. This result was confirmed in a following study showing that high levels of HER2 homodimers, which correlate with HER2 expression, could be predictive of increased trastuzumab activity (18).

About one third of HER2-positive tumors coexpress a truncated form of the receptor that lacks the extracellular domain, which is the binding site for trastuzumab (19). This C-terminal fragment of HER2, called p95HER2 for its molecular weight by Western blot analysis, is constitutively active, highly tumorigenic, and has been shown to correlate with intrinsic resistance to trastuzumab-based therapy in the metastatic setting (20–23). Recent data based on p95HER2 evaluation by immunohistochemistry in the neoadjuvant setting, however, showed the opposite trend, correlating the expression of this truncated form with response to trastuzumab-based treatment (24).

In this work, we aimed to study the correlation between HER2 and p95HER2 expressions and response to lapatinib, trastuzumab, or the combination of both in the neoadjuvant setting.

Materials and Methods

Patient population

p95HER2 and HER2 measurements were performed in primary tumors of 455 patients enrolled in the phase III neoadjuvant study NeoALTTO (13).

Statistical analysis

Univariate comparisons of levels of HER2 or p95HER2 in subgroups defined by clinicopathologic factors, or by pathologic complete response (pCR) status, were made using unpaired *t* tests applied to the log-transformed data. Multivariate analyses of pCR rate controlling for treatment arm and HR status (the only significantly predictive clinicopathologic factor) were done using logistic regression analysis. pCR was defined as absence of invasive tumor cells in the breast at time of surgery. Multivariate analyses of event-free survival (25) were done using Cox regression modeling. Analyses were done in R version 3.0.1 and SAS 9.3.

Quantitative HER2 assay (HERmark)

Total HER2 protein expression was quantified using the HERmark assay as previously described (26). The HERmark HER2 assay has been validated according to CLIA standards and is performed in a CAP-accredited central laboratory. The validation data have been published (27). Expected coefficients of variation (CV's) are approximately 20% and 98% concordance with centrally determined HER2 status has been demonstrated (26). A single FFPE slide with a minimum of 5 mm^2 tumor is required for the HERmark HER2 assay. Briefly, the method entails HER2 quantification through the release of a fluorescent tag conjugated to an HER2 mAb via a linker that is sensitive to singlet oxygen. The first antibody was paired with a biotinylated second HER2 mAb. An avidin-linked photosensitizer molecule produces singlet oxygen upon illumination with red light. Because of the short half-life of singlet oxygen, the tag is only cleaved when the two antibodies are bound to the same HER2 molecule. Fluorescence, quantified by capillary electrophoresis, was normalized to invasive tumor area on the FFPE tissue section to give final units of relative fluorescence/mm^2 tumor (RF/mm^2), a measure of average HER2 expression in the tumor. Measurements were normalized to cell line standards of known HER2 expression level.

Quantitative p95 assay (VeraTag)

P95HER2 was quantified using the VeraTag platform with a proprietary mAb specific for the active M611-CTF form of p95 as previously reported (28). This antibody does not detect HER2 proteolytically cleaved at A648, but may detect longer proteolytically processed HER2. Briefly, the p95 antibody was conjugated to a fluorescent tag via a linker that is sensitive to reduction by dithiothreitol (DTT). Following release by DTT, the fluorescence signal was quantified as described above. Similar to the HERmark assay, the p95 assay provides a measure of average tumor expression of p95. A single FFPE slide with a minimum of 10 mm^2 tumor is required for the p95 VeraTag assay. CV's for the p95 assay are similar to the HERmark assay.

Results

Correlation between p95HER2 and HER2 expressions

Sufficient tissue was available to measure p95HER2 in 281 cases (62%) and HER2 in 324 cases (71%; Fig. 1A). These patient populations were well balanced for hormone receptor (HR) status and treatment arms (Table 1). Moreover, their clinicopathologic characteristics and pCR rates were in line with the overall study population (Table 1).

Overall, in the 274 cases (60%) in which quantification of both markers was available, we found some degree of correlation (slope = 0.38; $R^2 = 0.27$; $P < 0.001$, Fig. 1B) between p95HER2 and HER2 expressions. However, in patients with HER2 levels above the median (100 RF/mm^2), the correlation with p95HER2 expression was more evident (slope = 0.53; $R^2 = 0.15$; $P < 0.001$, Fig. 1B), suggesting that p95HER2-positive tumors often coexpress high levels of HER2.

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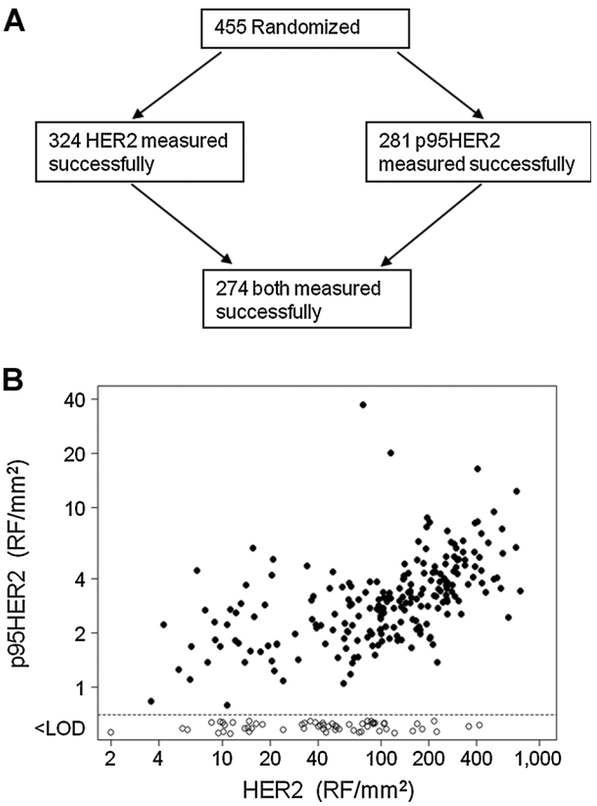

Figure 1.
Correlation between p95HER2 and HER2 expressions. A, scheme of samples available for either p95HER2 or HER2 quantification. B, correlation of tumors with different levels of p95HER2 and total HER2. p95 measurements below the limit of detection are indicated as "<LOD."

Correlation between expression of p95HER2 and HER2 and pathologic complete response in response to trastuzumab, lapatinib, or the combination

Next, we aimed to investigate whether p95HER2 or HER2 could predict pCR in patients treated with lapatinib, trastuzumab, or the combination of both agents. Because the distributions of p95HER2 and HER2 were strongly right-skewed, both variables were log transformed before further analysis, and logarithms to base 2 were used to ease interpretation of the ORs derived from logistic regression. For p95HER2 and HER2, we examined the relationship to pCR in multivariate models (Table 2, models A and B). The regression model relating p95HER2 to pCR rate showed a positive association in the trastuzumab-containing arms, but appears to be independent of p95HER2 levels in the lapatinib-alone arm (Table 2, model A). The regression model relating HER2 to pCR showed that pCR rate increases with increasing HER2 level, predominantly in the combination arm (Table 2, model B). Importantly, the OR for $\log_2$(HER2) in the combination arm [OR, 2.02; 95% confidence interval (CI), 1.42–2.87] was much greater than in the trastuzumab only arm (OR, 1.21; 95% CI, 0.93–1.57), implying that patients with the highest levels of HER2 expression in their tumor received the most benefit from the addition of lapatinib to trastuzumab. This is illustrated in a plot of predicted pCR versus HER2 using the coefficients for $\log_2$(HER2) from the model B (Fig. 2).

To explore whether the apparent effect of HER2 (measured by HERmark) was sustained in patients with higher expression levels measured by standard IHC testing, we repeated the analysis only in tumors with documented 3+ score by IHC. Of note, 324 patients were recorded as having IHC3$^+$ staining cells in their diagnostic biopsy. HER2 levels by HERmark were measured in 232 of these samples. The distribution of HER2 values in this subset is extremely similar to that in the complete trial (Supplementary Fig. S1). The logistic and Cox model regression results (Supplementary Table S1) are also very similar to those obtained from the full model. In both cases, the confidence intervals are widened, reflecting the reduced sample size, but the direction and magnitude of the HERmark and treatment arm effects are essentially unaltered (Fig. 2B shows the fitted model for comparison with Fig. 2A, illustrating the close similarity between the models).

We then fitted a logistic regression model containing both p95HER2 and HER2, to examine whether there was any evidence that the two antigens were "jointly predictive" of pCR. Akaike information criterion (AIC) values were calculated for models containing either p95HER, HER2, or both. We found that the

Table 1. Description of the patient population

	p95HER measured ($N = 281$)	HER2 measured ($N = 324$)	p95HER and HER2 measured ($N = 274$)	Randomized patients ($N = 455$)
HR status				
Positive	139 (49.5%)	166 (51.2%)	136 (49.6%)	232 (51.0%)
Negative	142 (50.5%)	158 (48.8%)	138 (50.4%)	223 (49.0%)
Tumor size				
≤5	174 (61.9%)	198 (61.1%)	171 (62.4%)	274 (60.2%)
>5	107 (38.1%)	126 (38.9%)	103 (37.6%)	181 (39.8%)
Nodal status at baseline				
N0/1	235 (83.6%)	275 (84.9%)	230 (83.9%)	383 (84.2%)
N2+	46 (16.4%)	49 (15.1%)	44 (16.1%)	72 (15.8%)
Planned surgery				
Not conservative	195 (69.4%)	226 (69.8%)	189 (69.0%)	325 (71.4%)
Conservative	86 (30.6%)	98 (30.2%)	85 (31.0%)	130 (28.6%)
Treatment				
L	97 (34.5%)	115 (35.5%)	94 (34.3%)	154 (33.8%)
T	93 (33.1%)	106 (32.7%)	90 (32.8%)	149 (32.7%)
L+T	91 (32.4%)	103 (31.8%)	90 (32.8%)	152 (33.4%)
pCR status				
No pCR	180 (64.1%)	210 (64.8%)	175 (63.9%)	295 (64.8%)
pCR	101 (35.9%)	114 (35.2%)	99 (36.1%)	160 (35.2%)

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Table 2. Multivariate models

Effect	*P*	pCR OR[a]	PFS HR[b]	95% CI[c]	Arm
Model A					
Treatment arm	0.24	0.64		0.33–1.24	L v T
		2.09		1.08–4.03	L+T v T
Hormone receptor negative	0.0008	2.49		1.46–4.25	
Tumor size	0.24	1.39		0.80–2.41	
Lymph node status	0.45	0.77		0.38–1.56	
Conservative plan	0.51	0.82		0.45–1.47	
Log_2(p95HER2) by Arm	0.08	0.97		0.69–1.37	L
		1.67		1.06–2.64	T
		1.60		1.11–2.31	L+T
Log_2(p95HER2)	0.006				
Model B					
Treatment arm	0.28	0.84		0.45–1.55	L v T
		2.89		1.54–5.44	L+T v T
Hormone receptor negative	0.003	2.18		1.30–3.66	
Tumor size	0.46	1.22		0.72–2.06	
Lymph node status	0.58	0.82		0.41–1.66	
Conservative plan	0.53	0.83		0.47–1.47	
Log_2(HER2) by Arm	0.06	1.30		0.97–1.75	L
		1.21		0.93–1.57	T
		2.02		1.42–2.87	L+T
Log_2(HER2)	<0.001				
Model C					
Treatment arm	0.26		1.20	0.68–2.12	L v T
			0.72	0.38–1.35	L+T v T
Hormone receptor negative	0.01		1.91	1.15–3.18	
Tumor size	0.049		0.62	0.38–1.00	
Lymph node status	0.29		0.72	0.39–1.32	
Conservative plan	0.10		0.60	0.33–1.10	
Log_2(p95HER2)	0.01		0.71[d]	0.54–0.93	
Model D					
Treatment arm	0.12		1.35	0.79–2.29	L v T
			0.74	0.41–1.34	L+T v T
Hormone receptor negative	0.05		1.60	1.00–2.56	
Tumor size	0.13		0.70	0.44–1.11	
Lymph node status	0.24		0.70	0.39–1.27	
Conservative plan	0.04		0.55	0.31–0.97	
Log_2(HER2)	0.01		0.67[d]	0.50–0.91	

[a]OR for logistic regression model relating pCR to clinicopathologic variables in models A and B.
[b]HR for Cox model analysis relating PFS to clinicopathologic variables in models C and D.
[c]95% confidence interval.
[d]HR of the interquartile range in p95HER2 or HER2.

model containing HER2 (and its interaction with the treatment arm) was preferred to either the model containing p95HER2 alone, or the model containing both, based on AIC.

Because HR status was found to be a strong determinant of pCR in this cohort (13), we examined the HR-negative and -positive groups separately. Expression of p95HER2 was mainly predictive for pCR in HR-positive patients treated with either trastuzumab or the combination of L+T. The ratio of geometric means in p95HER2 expression between HR-positive patients achieving pCR versus patients not achieving pCR was 1.0 (95% CI, 0.50–1.87; $P = 0.92$) for patients treated with lapatinib, 1.6 (95% CI, 1.0–2.71; $P = 0.05$) for patients treated with trastuzumab, and 2.1 (95% CI, 1.2–3.7; $P = 0.01$) for patients treated with L+T. In HR-negative patients, p95HER2 expression did not predict for pCR in any of the treatment arms (Fig. 3A). HER2 expression did not predict for pCR in the lapatinib and trastuzumab monotherapy arms in HR-negative patients and weakly predicted for pCR upon lapatinib or trastuzumab treatments as single agents in HR-positive patients, with ratios of geometric means of 1.7 (95% CI, 0.79–3.82; $P = 0.17$) and 1.9 (95% CI, 0.81–4.26; $P = 0.14$), respectively. However, HER2 expression strongly correlated with pCR in both HR-positive (ratio of 3.1; 95% CI, 1.61–5.81; $P = 0.001$) and HR-negative (ratio of 2.5; 95% CI, 1.44–4.20; $P = 0.001$) in patients treated with L+T (Fig. 3B).

Correlation between expression of p95HER2 and HER2 and PFS in response to trastuzumab, lapatinib, or the combination

To examine whether the levels of either p95HER2 or HER2 could predict for PFS in patients treated with lapatinib, trastuzumab, or L+T, we fit to the PFS data in Cox models containing log_2(p95HER2) or log_2(HER2; Table 2, models C and D). The limited number of PFS events precluded fitting HRs for log_2(p95HER2) or log_2(HER2) in individual arms. Longer PFS was associated with both increasing log_2(p95HER2) and increasing log_2(HER2). HER2 had a smaller HR (HR, 0.66; $P = 0.01$, model D) compared with p95HER2 (HR, 0.71; $P = 0.01$, model C).

Discussion

In this work, we showed that increasing HER2 protein expression was associated with increased odds to achieve pCR in patients treated with trastuzumab and the combination of trastuzumab and lapatinib. In particular, increasing HER2 levels positively correlated with increased benefit of adding

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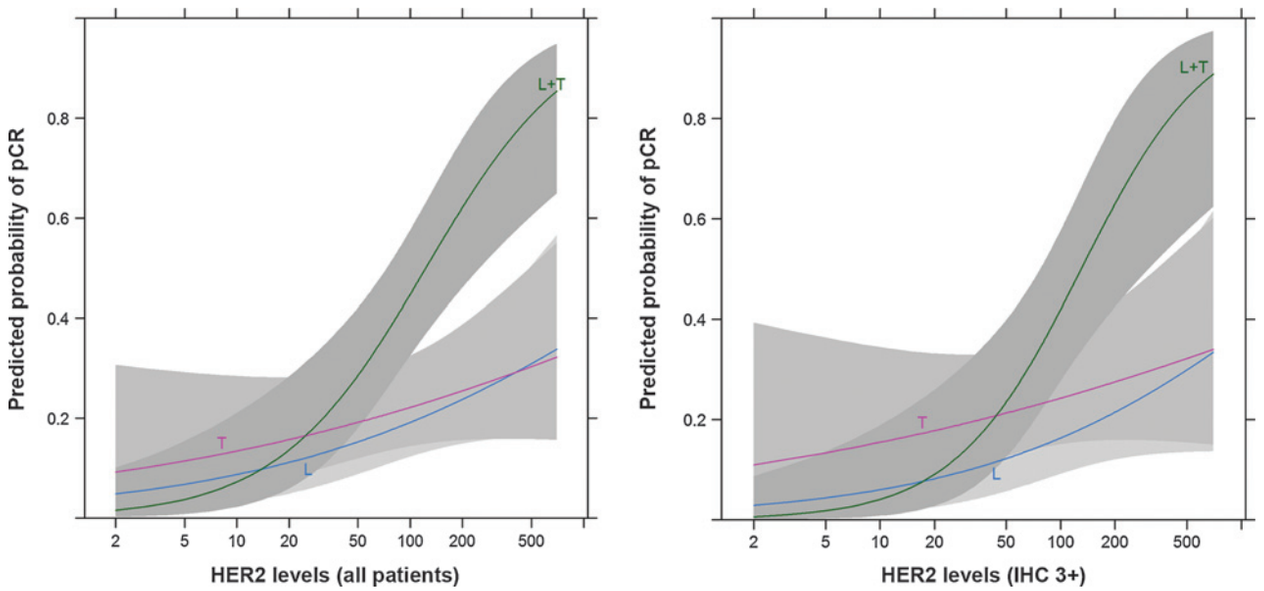

Figure 2.
Predicted pCR based on HER2 expression. Predicted pCR based on HER2 expression for all patients with HER2 measurement (A) and only in patients with IHC 3+ score by IHC (B).

lapatinib to trastuzumab when compared with trastuzumab alone. Moreover, we provide evidence that, in the neoadjuvant setting, the association between p95HER2 expression and response to anti-HER2 therapy is likely a consequence of the close association between p95HER2 and HER2 levels. These findings could explain, at least in part, the results obtained by Loibl and colleagues (24) showing that p95HER2 assessed by IHC was predictive of response to trastuzumab-based therapy in the neoadjuvant setting.

It is becoming clear that among "HER2-positive" tumors, there is a wide range of variability in terms of HER2 gene amplification and protein expression as measured by conventional semiquantitative methods such as the HercepTest. The possibility that a quantitative analysis of HER2 expression could improve prediction of response to anti-HER2 therapy has led to the evaluation of alternative tests that are quantitative for protein expression levels. For example, the HERmark assay has been shown to provide continuous, rather than semiquantitative, measurement of HER2 and its homodimer over a wide range of expression levels (16). In a retrospective study with 102 patients with HER2-positive metastatic breast cancer who had received trastuzumab-based therapy and in which reassessment of HER2 status was done by the HERmark assay, the authors showed that high HER2 expression, which was found in 87% and 14% of FISH-positive and -negative tumors, respectively, was significantly associated with better outcomes (17). Conversely, the outcome of patients whose tumors expressed lower levels of HER2 was worse, irrespective

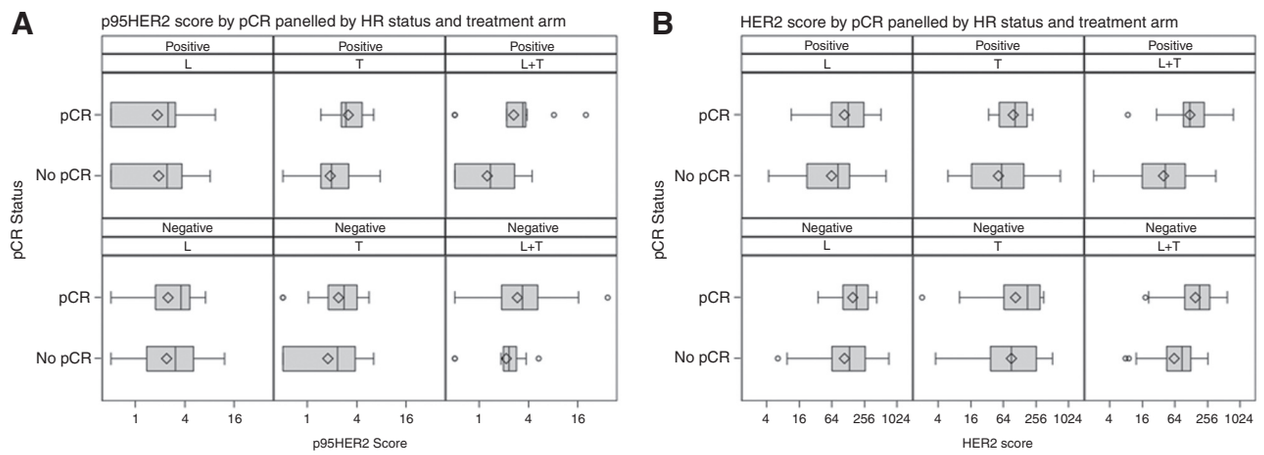

Figure 3.
Box-whisker plots for p95HER2 against pCR status split by treatment arm and HR status (A). Box-whisker plots for HER2 against pCR status split by treatment arm and HR status (B). Summary statistics calculated on log scale, then plotted on raw data scale.

Published OnlineFirst December 2, 2014; DOI: 10.1158/1078-0432.CCR-14-1824

of FISH status. These results are consistent with another study showing that high levels of HER2 homodimers were predictive of increased response to trastuzumab-based therapy (18).

HER2 phosphorylation (pHER2), considered as the activated state of the receptor, largely coincides with HER2 overexpression (29, 30). However, it was found that also a subgroup of HER2-negative tumors express detectable levels of pHER2 that correlate with relatively high HER3 phosphorylation (29). This finding confirms that HER2/HER3 dimerization is central for HER2 signaling and indirectly suggests that tumors with low levels of HER2 but high levels of pHER2 might still benefit from anti-HER2 therapy, perhaps including the dimerization inhibitor antibody pertuzumab. Yet, the results reported in literature regarding the role of pHER2 in predicting response to anti-HER2 therapy are contradictory. In fact, evidences in favor of positive (30), inverse (31), or no (32) association between pHER2 levels and clinical response to trastuzumab-based therapy have been described. Furthermore, inhibition of pHER2 following lapatinib measured in serial biopsies did not significantly correlate with clinical response (33). These discrepancies may be due in large part to the different techniques and antibodies used for pHER2 detection and the intrinsic heterogeneity of the different tumor specimens. In any case, it would be interesting to measure the levels of phospho-HER2 in our cohort of samples, and correlate these findings with clinical outcome.

The relationship between HER2 level and outcome has also been examined using HER2 mRNA. In GeparQuattro, HER2 mRNA was found to correlate with pCR, but only in the ESR1-positive subgroup (34). Similarly, in the analogous, albeit smaller, trastuzumab arm of the NeoALTTO study, HER2 protein expression trended toward a correlation with pCR in the HR-positive group, but there was no relationship between HER2 and pCR in the HR-negative group. In addition, a relationship between HER2 mRNA and disease-free survival was found in NSABP B-31, also with an interaction with ESR1 expression (35).

About 50% of clinically defined HER2-positive tumors fall into an HER2-enriched (HER2-E) mRNA subtype, which is characterized by increased expression of *FGFR4*, *EGFR*, *HER2*, and other genes within the *HER2* amplicon (i.e., *GRB7*; ref. 36). The remaining tumors belong predominantly to the luminal A or B mRNA subtypes and were found to have increased expression of "luminal genes" such as *GATA3*, *BCL2*, and *ESR1*. In a recent study conducted in patients who received either trastuzumab or lapatinib monotherapy (chemo free), we showed that patients with HER2-E tumors (and higher HER2 expression measured with HERmark) were significantly more likely to benefit from anti-HER2 therapy (37). In accordance, results from the TBCRC006 trial (38, 39) indicate that a significant fraction of HER2-positive patients respond to the combination of lapatinib and trastuzumab without the need of cytotoxic chemotherapy. In particular, this phenomenon seems to be more evident in the ER-positive subset when antihormonal therapy is added to dual HER2 blockade. Despite the fact that these data were obtained from a small cohort of patients, it is tempting to speculate that, perhaps, patients with high levels of HER2 and with greater odds to respond to the combination of trastuzumab and lapatinib are the ones that may achieve pCR without the need of concomitant chemotherapy.

In this neoadjuvant study, quantitative HER2 expression was found to be more determinative of outcome that p95HER2 expression. In contrast, in the metastatic setting, p95HER2 has been found to negatively correlate with PFS and OS (23). It is possible that this difference could be due to phenotypic differences in the primary and metastatic tumors, especially under selective pressure of drug treatment.

The role of pCR as a surrogate marker for long-term disease control is still a matter of debate. In particular, von Minckwitz and colleagues (40) reported that this is not the case in patients with HER2-positive ER-positive disease. In our work, we show that high levels of HER2 are predictive of pCR in response to dual HER2 blockade irrespective of the hormonal status of the tumors and that this translated in improved disease-free survival at 3 years.

An important control in our study is the correlation of HER2 levels and predicted pCR rate calculated analyzing only tumors with documented 3+ score by IHC. The main advantage of quantifying HER2 expression by HERmark among the homogeneous population with positive HER2 staining would be the identification of those patients that are more likely to respond to the combination of lapatinib and trastuzumab. This can have tangible clinical impact. First, given that dual HER2 blockade is often not well tolerated, we could spare patients with low HER2 expression from this therapeutic option. Second, it is tempting to speculate that the patients bearing tumors with high HER2 levels will achieve better long-term benefit (PFS), if treated with dual HER2 blockade.

In summary, our findings indicate that total levels of HER2 can predict benefit for the combination of L+T. The added value of HER2 quantification is indeed its predictive power. The HERmark assay can identify those patients from whom we should not expect dramatic responses from anti-HER2 therapy, and therefore potentially suitable for alternative therapeutic strategies. On the other hand, the same assay can identify those patients that will benefit from dual HER2 blockade upfront. Further studies to understand the impact of p95HER2 and HER2 expressions on disease-free and overall survival following anti-HER2 therapy are warranted.

Disclosure of Potential Conflicts of Interest

M. Scaltriti reports receiving speakers bureau honoraria from GlaxoSmithKline. J. Sperinde, A. Chenna, and J. Winslow are employees of LabCorp, Inc. N. Harbeck reports receiving speakers bureau honoraria from Roche, and is a consultant/advisory board member for GlaxoSmithKline and Roche. J. Cortes reports receiving speakers bureau honoraria from Celgene, Eisai, Novartis, and Roche, and is a consultant/advisory board member for Celgene and Roche. E. de Azambuja reports receiving speakers bureau honoraria from Roche, and is a consultant/advisory board member for GlaxoSmithKline. J. Baselga is a consultant/advisory board member for Genentech. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank Marta Guzman for technical assistance.

Grant Support

This work was funded by GlaxoSmithKline, European Research Council grant (AdG09 250244), Instituto de Salud Carlos III Intrasalud Grant (PSO9/00623), and Banco Bilbao Vizcaya Argentaria (BBVA) Foundation (Tumor Biomarker Research Program).

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Received July 16, 2014; revised November 12, 2014; accepted November 17, 2014; published OnlineFirst December 2, 2014.

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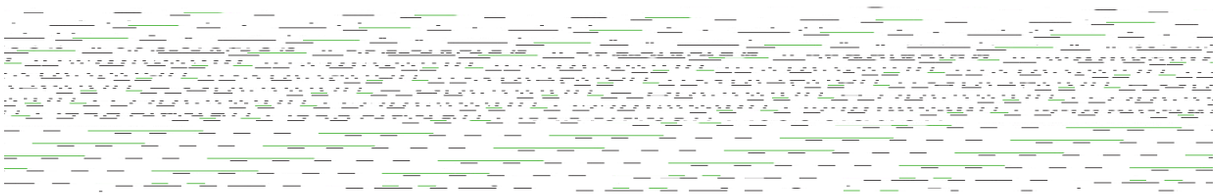
Published OnlineFirst December 2, 2014; DOI: 10.1158/1078-0432.CCR-14-1824



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Clin Cancer Res 2015;21:569-576. Published OnlineFirst December 2, 2014.