High HER2 expression correlates with response to the combination of lapatinib and trastuzumab

Maurizio Scaltriti¹, Paolo Nuciforo², Ian Bradbury³, Jeff Sperinde⁴, Dominique Agbor-Tarb³, Christine Campbell³, Ahmed Chenna⁴, John Winslow⁴, Violeta Serra⁵, Josep Lluis Parra⁵, Ludmila Prudkin², José Jimenez², Claudia Aura², Nadia Harbeck⁶, Lajos Pusztai⁷, Catherine Ellis⁸, Holger Eidtmann⁹, Joaquin Arribas⁵,13, Javier Cortes¹⁰, Evandro de Azambuja¹¹, Martine Piccart¹¹ and José Baselga¹,12.

¹Human Oncology & Pathogenesis Program (HOPP) and ¹²Breast Medicine Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, Box 20, New York, NY 10065.
²Molecular Oncology Laboratory, ⁵Experimental Therapeutics Group and ¹⁰Medical Oncology Department, Vall d’Hebron Institute of Oncology, Passeig Vall d’Hebron 119-129, Barcelona 08035, Spain.
³Frontier Science (Scotland) Ltd, Grampian View, Kincraig, Inverness-shire, PH21 1NA, Scotland.
⁴Clinical Research, Monogram Biosciences, Inc., 345 Oyster Point Blvd., South San Francisco, CA 94080.
⁶Breast Center, Department Obstetrics & Gynecology & CCC LMU, University of Munich, Marchioninistrasse 15, 81377 Munich, Germany
⁷Genetics and Genomics Program, Yale School of Medicine, 333 Cedar St, New Haven, CT 06520.
⁸Oncology R&D, GlaxoSmithKline, 1250 S. Collegeville Road, Collegeville, PA 19426.
⁹Department of Gynecology and Midwifery, University Hospital Kiel, Arnold-Heller-Straße 3, 24105 Kiel, Germany
¹¹Jules Bordet Institute, Bld de Waterloo, 121(7th Floor)1000 Brussels, Belgium.
¹³Catalan Institution for Research and Advanced Studies (ICREA)
Corresponding Author:

Maurizio Scaltriti, PhD
Human Oncology & Pathogenesis Program (HOPP)
Memorial Sloan Kettering Cancer Center
1275 York Avenue, Box 20
New York, NY 10065
Tel: 646-888-3519
Fax: 646-422-0247
E-mail: scaltrim@mskcc.org

Running title: HER2 expression and benefit from dual HER2 blockade

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

Conflicts of interest: MS received speaker honoraria from GlaxoSmithKline; JS, AC and JW are Monogram Biosciences employees. CE is a GlaxoSmithKline employee. EdA received honoraria from Roche, research funding from GlaxoSmithKline and is a consultant for Roche; NH received speaker honoraria from GlaxoSmithKline and Roche and is a consultant for GlaxoSmithKline and Roche; JC is a consultant for Roche; MP received honoraria from Roche; JB is in the advisory board of Genentech.
Statement of Translational Relevance

The range of expression of HER2 in tumors from HER2-positive breast cancer patients is extremely variable. In this manuscript 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.
Abstract

Purpose: Expression of p95HER2 has been associated with resistance to trastuzumab (T)-based therapy in metastatic breast cancer patients. 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 breast cancer patients.

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 pathological complete response (pCR) and progression-free survival (PFS) following lapatinib (L), 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%) where 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. In order to examine whether the levels of either p95HER2 or HER2 could predict for PFS in patients treated with L, T 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 L to T. HER2 expression is a stronger predictor of pCR and PFS than p95HER2 for response to L, T and, more significantly, L+T.
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: monoclonal antibodies binding to extracellular domains of the receptor (e.g. trastuzumab, pertuzumab, 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 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 HER2 positive breast cancer patients 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 HER2 positive breast cancer patients (10, 11). Recently, the antitumor activity of dual HER2 blockade (T in combination with either pertuzumab or L) 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) (15), there is a wide range of variability in terms of HER2-gene amplification and protein expression measured by the conventional semi-quantitative 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 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 T activity (18).
About one third of HER2 positive tumors co-express a truncated form of the receptor that lacks the extracellular domain, which is the binding site for T (19). This C-terminal fragment of HER2, called p95HER2 for its molecular weight by western blot, 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 expression 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 methods

Univariate comparisons of levels of HER2 or p95HER2 in subgroups defined by clinico-pathological factors, or by pathological 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 clinico-pathological 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 (EFS,(25)) were done using Cox regression modeling. Analyses were done in R version 3.1.0 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 CV’s are ~20% and 98% concordance with centrally determined HER2 status has been demonstrated (26). A single FFPE slide with a minimum of 5 mm² tumor is required for the HERmark HER2 assay. Briefly, the method entails HER2 quantification through the release of a fluorescent tag conjugated to a HER2 monoclonal antibody (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. Due to 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 / mm².
tumor (RF/mm²), 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 mm² 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 expression

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 clinico-pathological characteristics and pCR rates were in line with the overall study population (Table 1).

Overall, in the 274 cases (60%) where 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 expression. 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 co-express high levels of HER2.

Correlation between expression of p95HER2 and HER2 and pathological 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 odds ratios 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 odds ratio for $\log_2$(HER2) in the combination arm (OR = 2.02; 95%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 pCR versus HER2 using the coefficients for $\log_2$(HER2) from Model B (Fig. 2).
In order 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. 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 HER2mark 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 to 2A, illustrating the close similarity between the models).

We then fitted a logistic regression model containing both p95HER2 and HER2, to examine if 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 model containing HER2 (and its interaction with treatment arm) was preferred to either the model containing p95HER2 alone, or the model containing both based on AIC.

Since 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 T 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 to 1.87, p=0.92) for patients treated with L, 1.6 (95%CI 1.0 to 2.71, p=0.05) for patients treated with T and 2.1 (95%CI 1.2 to 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 L and T monotherapy arms in HR-negative patients and weakly predicted for pCR upon L or T treatments as single agents in HR-positive patients, with ratios of geometric means of 1.7 (95%CI 0.79 to 3.82, p=0.17) and 1.9 (95%CI 0.81 to 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 to 5.81, p=0.001) and HR-negative (ratio of 2.5, 95%CI 1.44 to 4.20, p=0.001) in patients treated with L+T (Fig. 3B).
Correlation between expression of p95HER2 and HER2 and progression-free survival in response to trastuzumab, lapatinib or the combination

In order to examine whether the levels of either p95HER2 or HER2 could predict for progression-free survival (PFS) in patients treated with L, T 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 hazard ratios 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 hazard ratio (HR = 0.66, p = 0.01, Model D) compared to 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 lapatinib to trastuzumab when compared to 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 et al. showing that p95HER2 assessed by IHC was predictive of response to trastuzumab-based therapy in the neoadjuvant setting (24).

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 semi-quantitative 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 HER2 positive metastatic breast cancer patients who had received trastuzumab-based therapy and where 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 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.
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 utilized 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 towards a correlation with pCR in the hormone receptor positive group, but there was no relationship between HER2 and pCR in the hormone receptor negative group. Additionally, 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 a 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. $GRB7$) (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 anti-hormonal 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 the current 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 matter of debate. In particular, von Minckwitz and colleagues reported that this is not the case in patients with HER2-positive ER-positive disease (40). In our work we show that high levels of HER2 are predictive of pCR in response to dual HER-2 blockade irrespective of the hormonal status of the tumors and that this translated in improved disease-free survival at three 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 expression on disease-free and overall survival following anti-HER2 therapy are warranted.

Acknowledgments

We thank Marta Guzman for technical assistance.
References


Figure legends

Figure 1. Correlation between p95HER2 and HER2 expression. 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".

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

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

Supplementary Figure legends

Figure S1. Distribution of HER2 expression (measured by HERmark) in all patients (left) and in 3+ IHC cohort patients (right). All patient: n=324; IHC3+: n=232.
Table 1. Description of the patient population.

<table>
<thead>
<tr>
<th></th>
<th>p95HER measured (N=281)</th>
<th>HER2 measured (N=324)</th>
<th>p95HER and HER2 measured (N=274)</th>
<th>Randomized patients (N=455)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>139 (49.5%)</td>
<td>166 (51.2%)</td>
<td>136 (49.6%)</td>
<td>232 (51.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>142 (50.5%)</td>
<td>158 (48.8%)</td>
<td>138 (50.4%)</td>
<td>223 (49.0%)</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=5</td>
<td>174 (61.9%)</td>
<td>198 (61.1%)</td>
<td>171 (62.4%)</td>
<td>274 (60.2%)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>107 (38.1%)</td>
<td>126 (38.9%)</td>
<td>103 (37.6%)</td>
<td>181 (39.8%)</td>
</tr>
<tr>
<td><strong>Nodal status at baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0/1</td>
<td>235 (83.6%)</td>
<td>275 (84.9%)</td>
<td>230 (83.9%)</td>
<td>383 (84.2%)</td>
</tr>
<tr>
<td>N2+</td>
<td>46 (16.4%)</td>
<td>49 (15.1%)</td>
<td>44 (16.1%)</td>
<td>72 (15.8%)</td>
</tr>
<tr>
<td><strong>Planned surgery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Conservative</td>
<td>195 (69.4%)</td>
<td>226 (69.8%)</td>
<td>189 (69.0%)</td>
<td>325 (71.4%)</td>
</tr>
<tr>
<td>Conservative</td>
<td>86 (30.6%)</td>
<td>98 (30.2%)</td>
<td>85 (31.0%)</td>
<td>130 (28.6%)</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>97 (34.5%)</td>
<td>115 (35.5%)</td>
<td>94 (34.3%)</td>
<td>154 (33.8%)</td>
</tr>
<tr>
<td>T</td>
<td>93 (33.1%)</td>
<td>106 (32.7%)</td>
<td>90 (32.8%)</td>
<td>149 (32.7%)</td>
</tr>
<tr>
<td>L+T</td>
<td>91 (32.4%)</td>
<td>103 (31.8%)</td>
<td>90 (32.8%)</td>
<td>152 (33.4%)</td>
</tr>
<tr>
<td><strong>pCR (Pathological Complete Response) status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pCR</td>
<td>180 (64.1%)</td>
<td>210 (64.8%)</td>
<td>175 (63.9%)</td>
<td>295 (64.8%)</td>
</tr>
<tr>
<td>pCR</td>
<td>101 (35.9%)</td>
<td>114 (35.2%)</td>
<td>99 (36.1%)</td>
<td>160 (35.2%)</td>
</tr>
</tbody>
</table>
Table 2. Multivariate models

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-value</th>
<th>pCR OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PFS HR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment Arm</td>
<td>0.24</td>
<td>0.64</td>
<td>0.33 – 1.24</td>
<td>L v T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.09</td>
<td>1.08 – 4.03</td>
<td>L+T v T</td>
</tr>
<tr>
<td>Hormone Receptor Negative</td>
<td>0.0008</td>
<td>2.49</td>
<td>1.46 – 4.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Size</td>
<td>0.24</td>
<td>1.39</td>
<td>0.80 – 2.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph Node Status</td>
<td>0.45</td>
<td>0.77</td>
<td>0.38 – 1.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative Plan</td>
<td>0.51</td>
<td>0.82</td>
<td>0.45 – 1.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log&lt;sub&gt;2&lt;/sub&gt;(p95HER2) by Arm</td>
<td>0.08</td>
<td>0.97</td>
<td>0.69 – 1.37</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.67</td>
<td>1.06 – 2.64</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.60</td>
<td>1.11 – 2.31</td>
<td>L+T</td>
</tr>
<tr>
<td>Log&lt;sub&gt;2&lt;/sub&gt;(p95HER2)</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment Arm</td>
<td>0.28</td>
<td>0.84</td>
<td>0.45 – 1.55</td>
<td>L v T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.89</td>
<td>1.54 – 5.44</td>
<td>L+T v T</td>
</tr>
<tr>
<td>Hormone Receptor Negative</td>
<td>0.003</td>
<td>2.18</td>
<td>1.30 – 3.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Size</td>
<td>0.46</td>
<td>1.22</td>
<td>0.72 – 2.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph Node Status</td>
<td>0.58</td>
<td>0.82</td>
<td>0.41 – 1.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative Plan</td>
<td>0.53</td>
<td>0.83</td>
<td>0.47 – 1.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log&lt;sub&gt;2&lt;/sub&gt;(HER2) by Arm</td>
<td>0.06</td>
<td>1.30</td>
<td>0.97 – 1.75</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.21</td>
<td>0.93 – 1.57</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.02</td>
<td>1.42 – 2.87</td>
<td>L+T</td>
</tr>
<tr>
<td>Log&lt;sub&gt;2&lt;/sub&gt;(HER2)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment Arm</td>
<td>0.26</td>
<td>1.20</td>
<td>0.68 – 2.12</td>
<td>L v T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.72</td>
<td>0.38 – 1.35</td>
<td>L+T v T</td>
</tr>
<tr>
<td>Hormone Receptor Negative</td>
<td>0.01</td>
<td>1.91</td>
<td>1.15 – 3.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Size</td>
<td>0.049</td>
<td>0.62</td>
<td>0.38 – 1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph Node Status</td>
<td>0.29</td>
<td>0.72</td>
<td>0.39 – 1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative Plan</td>
<td>0.10</td>
<td>0.60</td>
<td>0.33 – 1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log&lt;sub&gt;2&lt;/sub&gt;(p95HER2)</td>
<td>0.01</td>
<td>0.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.54 – 0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment Arm</td>
<td>0.12</td>
<td>1.35</td>
<td>0.79 – 2.29</td>
<td>L v T</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.74</td>
<td>0.41 – 1.34</td>
<td>L+T v T</td>
</tr>
<tr>
<td>Hormone Receptor Negative</td>
<td>0.05</td>
<td>1.60</td>
<td>1.00 – 2.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Size</td>
<td>0.13</td>
<td>0.70</td>
<td>0.44 – 1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph Node Status</td>
<td>0.24</td>
<td>0.70</td>
<td>0.39 – 1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conservative Plan</td>
<td>0.04</td>
<td>0.55</td>
<td>0.31 – 0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log&lt;sub&gt;2&lt;/sub&gt;(HER2)</td>
<td>0.01</td>
<td>0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50 – 0.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Odds ratio for logistic regression model relating pCR to clinicopathological variables in Models A and B.  
<sup>b</sup>Hazard ratio for Cox Model analysis relating PFS to clinicopathological variables in Models C and D.  
<sup>c</sup>95% confidence interval.  
<sup>d</sup>Hazard ratio of the interquartile range in p95HER2 or HER2.
Figure 1

A

455 Randomized

324 HER2 measured successfully

281 p95HER2 measured successfully

274 both measured successfully

B

Graph showing the relationship between p95HER2 (RF/mm²) and HER2 (RF/mm²) with points scattered across the graph. The x-axis represents HER2 (RF/mm²) ranging from 2 to 1000, and the y-axis represents p95HER2 (RF/mm²) ranging from <LOD to 40. The graph includes a dashed line at the LOD level.
Figure 2

The graphs illustrate the predicted probability of pCR (pathologic complete response) for HER2 levels in all patients and HER2 levels (IHC 3+) groups. The graph on the left shows the predicted probability of pCR for HER2 levels (all patients) with different treatment regimens (L, T, L+T) indicated by different lines and shaded areas representing the confidence intervals. The graph on the right shows a similar trend for HER2 levels (IHC 3+).
High HER2 expression correlates with response to the combination of lapatinib and trastuzumab

Maurizio Scaltriti, Paolo Nuciforo, Ian Bradbury, et al.

Clin Cancer Res  Published OnlineFirst December 2, 2014.

Updated version Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-1824

Supplementary Material Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2014/12/03/1078-0432.CCR-14-1824.DC1

Author Manuscript Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.