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p95HER2 methionine 611 carboxy-terminal fragment is predictive of trastuzumab adjuvant treatment benefit in the FinHer trial

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Running title: p95HER2 predictive of adjuvant trastuzumab benefit

Potential Conflicts Disclosure: J Sperinde, W Huang, A Chenna, J Winslow, YS Lie and CJ Petropoulos are employees of Monogram Biosciences and may own stock in Laboratory Corporation of America Holdings. H Joensuu has a co-appointment with Orion Pharma and owns stock from Sartar Therapeutics, Faron Pharmaceuticals and Orion Pharma. All remaining authors have declared no conflicts of interest.

Statement of significance: In the adjuvant setting, elevated p95HER2 expression is prognostic for poor outcomes in the absence of trastuzumab treatment and is predictive of favorable outcomes in response to chemotherapy plus trastuzumab treatment.
Abstract

Purpose: Expression of p95HER2 (p95), a truncated form of the HER2 receptor, which lacks the trastuzumab binding site but retains kinase activity, has been reported as a prognostic biomarker for poor outcomes in trastuzumab-treated HER2-positive metastatic breast cancer. The impact of p95 expression on trastuzumab treatment efficacy in early HER2-positive breast cancer is less clear. In the current study, p95 was tested as a predictive marker of trastuzumab treatment benefit in the HER2-positive subset of the FinHer adjuvant phase III trial.

Experimental Design: In the FinHer trial, 232 HER2-positive early breast cancer patients were randomized to receive chemotherapy plus 9-weeks of trastuzumab or no trastuzumab treatment. Quantitative p95 protein expression was measured in formalin-fixed paraffin-embedded samples using the p95 VeraTag® assay (Monogram Biosciences), specific for the M611 form of p95. Quantitative HER2 protein expression was measured using the HERmark® assay (Monogram Biosciences). Distant disease-free survival (DDFS) was used as the primary outcome measure.

Results: In the arm receiving chemotherapy only, increasing log_{10}(p95) correlated with shorter DDFS (HR=2.0; \( P = .02 \)). In the arm receiving chemotherapy plus trastuzumab (\( N=95 \)), increasing log_{10}(p95) was not correlated with a shorter DDFS. In a combined analysis of both treatment arms, high breast tumor p95 content was significantly correlated with trastuzumab treatment benefit in multivariate models (interaction \( P = .01 \)).

Conclusions: p95 expression levels were prognostic in the chemotherapy-alone arm and predictive of trastuzumab treatment benefit in FinHer. These results warrant further investigation of p95 as a predictive marker of trastuzumab treatment benefit in the adjuvant setting.
**Statement of translational relevance:** Elevated expression of p95HER2, the M611 carboxy-terminal fragment (CTF) of HER2, is a prognostic indicator of poor outcome in metastatic breast cancer. Measurement of p95HER2 in the FinHer adjuvant clinical trial provided the first evidence of the predictive power of p95HER2 comparing a trastuzumab-treated group vs. a control group not treated with trastuzumab. In the adjuvant setting, elevated p95HER2 expression is prognostic for poor outcomes in the absence of trastuzumab treatment and is predictive of favorable outcomes in response to chemotherapy plus trastuzumab treatment.
Introduction

p95HER2 (p95) is a truncated form of the HER2 receptor which lacks the extracellular domain and binding sites for trastuzumab and pertuzumab. Although several carboxy-terminal fragments (CTF) of HER2 have been described, the form with methionine 611 at its N-terminus (M611CTF) appears to be the most biologically relevant [1, 2], which is attributable to the covalent homodimerization of unpaired cysteines near the N-terminus. M611CTF enhances cell migration and invasion [3, 4], is more potent than full-length HER2 in supporting tumor formation [2, 3], promotes metastasis [5] and reduces estrogen receptor expression [6], potentially reducing the effectiveness of anti-estrogen therapies.

p95 is prognostic for poor outcome in HER2-positive patients receiving trastuzumab-based therapy in the metastatic setting [7-11]. However, p95 expression can enhance sensitivity to chemotherapy in vitro and in patient-derived xenograft models [12], raising the possibility that high p95 expression may be predictive of better outcomes in patients receiving concurrent chemotherapy and trastuzumab therapy in the adjuvant setting prior to chemotherapy resistance. In fact, in the neoadjuvant NeoALTTO trial, increasing p95 expression correlated with better pathological complete response (pCR) in the trastuzumab + chemotherapy arm, although this was attributed to higher HER2 expression in tumors expressing high p95 levels [13].

The clinical significance of breast tumor p95 expression is not well-defined in early breast cancer. The goal of the current study was to determine whether p95 is a predictive biomarker of treatment benefit for trastuzumab plus chemotherapy in the adjuvant setting among HER2-positive cancer patients within the FinHer phase III clinical trial [14, 15]. To our knowledge, this is the first study to correlate p95 expression with the degree of benefit of adding trastuzumab to chemotherapy in early breast cancer.
Materials and Methods

Patients

The results of the FinHer trial (ISRCTN76560285) have been reported previously [14, 15]. Briefly, 1,010 women diagnosed with early breast cancer were randomized to three 21-day cycles of either docetaxel or vinorelbine followed by three 21-day cycles of fluorouracil, epirubicin and cyclophosphamide. Those with HER2-positive disease as determined by immunohistochemistry (IHC) and confirmed by chromogenic in situ hybridization (CISH) were randomized to receive or not receive 9 weeks of trastuzumab. Chemotherapy arms were combined for the current analysis, but chemotherapy type was included either by stratification or as an independent variable. Out of the 232 patients who had HER2-positive cancer, 192 (82.8%) had sufficient formalin-fixed paraffin-embedded (FFPE) breast tumor tissue to measure HER2 and p95, and were included in the analysis (Table 1 and Figure S1). The set of 192 cases is likely an unbiased representation of the entire set since the remaining 40 cases were not included only because sufficient tissue was not available for testing. Final follow up for distant disease-free survival (DDFS) was used as the outcome measure [15]. The study protocol was approved by an Institutional Review Board (an ethics committee) of the Helsinki University Hospital (HUS113/13/03/02/09). The FinHer trial participants provided written informed consent before study entry.

Quantitative HER2 Measurements

HER2 protein expression was quantified using the HERmark assay, a CLIA/CAP validated assay performed in a clinical reference laboratory (Monogram Biosciences, South San Francisco, CA), as described previously [16-18]. Two HER2 antibodies bound to the same HER2 molecule enable the release of a fluorescent tag that is quantified by capillary electrophoresis [18]. In
capillary electrophoresis, the fluorescence of the released tag from the tumor is compared to the fluorescence of an internal standard, and their ratio (relative fluorescence, RF) is then normalized to the tumor area in the FFPE sample to generate units of RF/mm². The HERmark results in units of RF/mm² are proportional to the protein content targeted with the antibodies in the sample. Multiple cell line controls were included in each batch as standards for batch-to-batch normalization. Analytical cutoffs aligned to central HER2 determination were determined prior to the current study to define HERmark negative values (HER2 < 10.5 RF/mm²) and HERmark positive values (HER2 > 17.8 RF/mm²) with equivocal values defined as 10.5 RF/mm² ≤ HER2 ≤ 17.8 RF/mm² (supplementary Figure S2). These cutoffs were assigned based on the <5th percentile of centrally determined HER2-positives and the >95th percentile of centrally determined HER2-negatives, respectively, based on a reference database of 1,090 breast cancer patient samples. An established clinical cutoff of 13.8 RF/mm² that best discriminates favorable versus unfavorable treatment outcomes for patients receiving trastuzumab-based therapy [19] was used in all clinical analyses in the current study to define HER2-positivity by the HERmark assay.

Quantitative p95 Measurements

The VeraTag p95 assay, which utilizes the proprietary clone D9 p95 monoclonal antibody was previously described [8]. D9 specifically recognizes the highly active M611-HER2-CTF form of truncated HER2. Steric interference likely blocks D9 binding to full length HER2 [8]. D9 binding of p95 in formalin-fixed paraffin-embedded (FFPE) tumor sections was detected by a secondary antibody conjugated to a fluorescent VeraTag reporter molecule by a disulfide linkage. The VeraTag reporter was released by disulfide reduction and quantified by capillary electrophoresis. Similar to the HERmark assay, RF was normalized to tumor area to generate
units of RF/mm². Multiple reference cell lines were evaluated within each assay batch to enable batch-to-batch normalization. A pre-specified p95 ≥ 2.8 RF/mm² cutoff, derived from an independent training set [8] and verified in an independent validation set [9], was used to define p95-positivity in the current study.

**Statistical Methods**

The Mann-Whitney U test was used to calculate $P$-values for differences in distributions of p95 or HER2 in hormone receptor positive vs. negative subgroups. The Kaplan-Meier method and Cox proportional hazards models were used to assess correlations of patient outcomes with HER2, p95 and other clinical variables. DDFS was used as the primary outcome measure, defined as the time period from the date of randomization to the date of first cancer recurrence outside of the ipsilateral locoregional region or to death whenever death occurred before distant recurrence, censoring patients who were alive without distant recurrence on their last follow-up date [15]. In Cox analyses with interaction terms including trastuzumab and either HER2 or p95, the hazard ratio for trastuzumab treatment was calculated at the median HER2 or p95 level by normalizing the HER2 and p95 values by their respective median. The predictive power of HER2 or p95 was determined by $P < 0.05$ of the biomarker-trastuzumab interaction term in a multivariate Cox model.

**Results**

**p95HER and HER2 expression**

In all of the 192 cancers, HER2-positivity had been confirmed using CISH testing [15]. The fraction of HERmark HER2-negative patients was not statistically different ($P = 0.29$) from the
fraction of HER2 IHC 0 and 1+ patients as determined by central laboratory HER2 IHC retesting (Table 1).

HER2 expression was higher in the steroid hormone receptor (HR)-negative subset ($P = .01$) as previously reported [20] (Figure 1a). p95 levels were also higher in the HR-negative subset ($P < .001$), consistent with previous reports [9] (Figure 1b). Correspondingly, the distribution of p95 and HER2 for the HR-negative subset was shifted towards higher p95 and HER2, yielding 55% of patients above the p95 cutoff for HR-negative patients versus 32% for HR-positive patients (Figure 1c and 1d). For the subset above the HERmark clinical cutoff, 60% of the HR-negative patients were above the p95 cutoff, and 38% of the HR-positive patients were above the p95 cutoff. A weak correlation was observed between $\log_{10}(p95)$ and $\log_{10}(HER2)$ values ($R^2 = 0.28; P < .001$). This correlation was stronger in the HR-positive subset ($R^2 = 0.38; P < .001$) than in the HR-negative subset ($R^2 = 0.14; P < .001$).

**Prognostic correlation of HER2 and p95 with DDFS**

Correlations of HER2 and p95 expression levels with DDFS in the subsets of patients who were treated with chemotherapy alone versus chemotherapy plus trastuzumab were examined using stratification based on hormone receptor status, nodal status and chemotherapy type (Table 2). For these analyses, the two chemotherapy arms (docetaxel and vinorelbine) for each trastuzumab allocation group were combined. In the chemotherapy-only arms, tumor HER2 content was not associated with DDFS ($P = .30$), while increasing p95 was correlated with shorter DDFS (HR = $2.0/\log; P = .02$). In the chemotherapy plus trastuzumab arms, neither HER2 ($P = .93$) nor p95 ($P = .19$) was associated with DDFS. The observation that p95 correlated with DDFS in the absence of trastuzumab treatment, but not in the presence of
trastuzumab treatment, suggested that p95 expression levels may be predictive of the benefit of adding trastuzumab to chemotherapy.

**Predictive correlation of HER2 and p95 with trastuzumab benefit**

A test for biomarker-treatment interaction was performed for HER2 and p95 (Table 3) to determine whether HER2 or p95 expression was predictive of trastuzumab benefit. Important clinical variables were included in the multivariate models, including nodal status and hormone receptor status. Chemotherapy arm was also included as an independent variable. Breast tumor HER2 content was not predictive of trastuzumab benefit \( (P = .35) \); however, high tumor p95 content was predictive of trastuzumab benefit \( (P = .01) \).

Kaplan-Meier analysis was used to illustrate the difference in the hazard ratios of treatment arms with or without trastuzumab in the p95-negative and p95-positive subgroups (Figure 2). Patient groups with p95-negative tumors exhibited no differences in DDFS when treated with chemotherapy alone or chemotherapy plus trastuzumab \( (HR = 0.84; P = .66) \). In contrast, p95-positive patient groups had the shortest DDFS in the absence of adjuvant trastuzumab treatment, yet received the most benefit from trastuzumab \( (HR = 0.32; P = .03) \). The results remained essentially similar when the time to any recurrence was used as the end point in place of DDFS (data not shown).

The tests for interaction of trastuzumab with p95 were repeated with selected subsets of patients to determine which factors, if any, had the strongest influence on the predictive association of p95 with trastuzumab benefit. Separate interaction tests for hormone receptor negative and positive subsets demonstrated that a high breast cancer p95 content was highly predictive of trastuzumab benefit in the hormone receptor negative subset \( (P = .009) \) in contrast to the hormone receptor positive subset \( (P = .90) \) (Table 4). High p95 also appeared more
predictive of trastuzumab benefit in the docetaxel arm ($P = .02$) as opposed to the vinorelbine arm ($P = .52$) (Supplementary Table 1).

**Discussion**

p95 was first described as a correlate of known poor prognostic markers in breast cancer [21, 22]. Gradually, the entity discovered as p95 was revealed to be a family of HER2 CTFs varying in length and oncogenic activity [1, 2, 23, 24]. Generally, HER2 CTF’s truncated below the transmembrane domain of HER2 are most abundant yet biologically inactive, while those retaining the transmembrane domain exhibit the most potent activity. Specific antibodies against M611CTF, the most potent HER2 CTF, have enabled specific detection of M611CTF in the background of abundant inactive CTF’s [6, 8].

The first clinical study to characterize p95 expression in breast cancer before the era of trastuzumab treatment found that high p95 expression was an indicator of poor prognosis, and that p95 expression was largely responsible for the poor prognosis associated with HER2 overexpression [25]. Our observations reported here are consistent with these initial findings: p95 expression is prognostic for shorter DDFS in the arms receiving chemotherapy without trastuzumab, while HER2 expression is not correlated with DDFS (Table 2).

Although p95 is a prognostic marker of poor outcome in trastuzumab-treated metastatic breast cancer [7-11], p95 can be a favorable prognostic marker in early breast cancer [13, 26]. This seemingly paradoxical finding may be related to sensitivity to chemotherapy [12] in early breast cancer that may be lost in previously treated metastatic breast cancer. In the current study, we only observed a weak trend supporting a correlation between increasing p95 expression and longer DDFS ($HR = 0.58$/log; $P = .19$), possibly due to the low event rate in the adjuvant setting. Interestingly, the predictive power of p95 expression may be dependent on the type of
chemotherapy administered concurrently with trastuzumab. P95 was predictive of trastuzumab benefit in the docetaxel-containing arms but not the vinorelbine-containing arms (Supplementary Table 1). However, this finding may be influenced by the reduced effectiveness of vinorelbine as compared with docetaxel in combination with trastuzumab [15] or the smaller number of events in this subset analysis.

The current study is the first test of p95 as a predictive marker of trastuzumab treatment benefit. The hypothesis that p95 may be predictive of trastuzumab benefit can be built on findings that p95 is a marker of poor prognosis in the absence of trastuzumab treatment [25] and that p95 may be a marker of increased pCR in trastuzumab-treated neo-adjuvant breast cancer [13, 26]. In the current study we found that high p95 expression is predictive of trastuzumab benefit in adjuvant breast cancer in a multivariable Cox model (Table 3) based on the significant interaction $P$-value ($P = .01$), while breast cancer HER2 content was not predictive ($P = .30$) in the present cohort of patients where all cancers were HER2-positive by CISH.

HER2-directed tyrosine kinase inhibitors (TKI’s) can suppress p95-mediated signaling [7, 27]. Lapatinib, a TKI that inhibits HER2 and HER1, is approved for the second line treatment of metastatic HER2-positive breast cancer following a trastuzumab-containing treatment. More recently, second line lapatinib use was found inferior to trastuzumab emtansine (T-DM1) [28], likely relegating lapatinib to third line treatment. Although the NeoALTTO study showed a benefit of adding lapatinib to trastuzumab in the neoadjuvant setting [29], some other Phase III neoadjuvant trials reported only non-significant trends toward benefit with the combination [30, 31, 32]. In the ALTTO adjuvant trial, there was a non-significant trend supporting the benefit of adding lapatinib to trastuzumab [33]. Taken together, these trial results suggest that HER2-targeting TKI’s might be most effective when used in a selected subset of the HER2-positive breast cancer patients. To this end, we have identified a subset of HER2-positive breast cancer
with high p95 expression that exhibited the most benefit from trastuzumab treatment. In NeoALTTO, high tumor p95 content correlated with a benefit of adding lapatinib to trastuzumab; tumors with high p95 expression also had the highest HER2 expression [13]. As new HER2-targeting TKI’s emerge, such as tucatinib and neratinib, further investigations exploring the utility of quantitative p95 and HER2 expression as predictive indicators of response to dual HER-2 directed therapy are warranted.

We note that the size of the subset of patients with HER2-positive cancer was relatively small in the FinHer trial, thus limiting the statistical power of this study. Despite this limitation, we found a statistically significant association between tumor p95 content and DDFS, suggesting that the oncogenic impact of p95 expression is strong. In the FinHer trial, trastuzumab administration was limited to 9 weeks concomitant with chemotherapy, while the current standard duration of trastuzumab administration is 12 months. Therefore, further study is warranted to investigate the predictive correlations between tumor p95 expression levels and survival in a setting where adjuvant trastuzumab is administered for a longer duration.

In summary, we conclude that patients with high breast tumor p95 levels experience unfavorable clinical outcomes relative to patients with low tumor p95 levels when the patients are treated with surgery and adjuvant chemotherapy, but not when the systemic treatment includes trastuzumab. This finding suggests that high tumor p95 content is a predictive biomarker for the efficacy of adjuvant trastuzumab.

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Disclosures

J Sperinde, W Huang, A Chenna, J Winslow, YS Lie and CJ Petropoulos are employees of Monogram Biosciences and may own stock in Laboratory Corporation of America Holdings. H Joensuu has a co-appointment with Orion Pharma and owns stock from Sartar Therapeutics, Faron Pharmaceuticals and Orion Pharma. All remaining authors have declared no conflicts of interest.

References


Figure Legends:

Figure 1. p95 and HER2 distributions according to hormone receptor status. HER2 reference lines (dashed lines) correspond to HERmark negative, equivocal and positive analytical cutoffs. The p95 reference line is the prognostic clinical cutoff for trastuzumab-treated metastatic breast cancer [8, 9]. HER2 protein expression by hormone receptor status (A). p95 HER2 protein expression by hormone receptor status (B). p95 versus HER2 expression in the HR-negative subset (C). p95 versus HER2 expression in the HR-positive subset (D). The solid boxes denote the median and the upper and the lower quartiles.

Figure 2. Kaplan-Meier plots of DDFS by adjuvant treatment containing versus not containing trastuzumab for p95-negative tumors (A) and p95-positive tumors (B).
### Table 1. Patient characteristics

<table>
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<tr>
<th>Characteristic</th>
<th>Category</th>
<th>N</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>HER2 CISH</td>
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<td>192</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>126</td>
<td>66</td>
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<tr>
<td>Central HER2 IHC retest</td>
<td>2+</td>
<td>53</td>
<td>28</td>
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<tr>
<td></td>
<td>1+</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
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<td>154</td>
<td>80</td>
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<td></td>
<td>equivocal</td>
<td>14</td>
<td>7</td>
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<td>45</td>
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<tr>
<td></td>
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<td>99</td>
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<td></td>
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<td>Hormone receptor&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>negative</td>
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<td>Adjuvant trastuzumab administered</td>
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<td>51</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>95</td>
<td>49</td>
</tr>
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HER2, human epidermal growth factor receptor 2, CISH, chromogenic in situ hybridization; ER, estrogen receptor; PR, progesterone receptor

<sup>a</sup>Cutoffs of 10.5 and 17.8 for the HERmark HER2 assay and 2.8 for the p95 assays were applied as described in the Methods section.

<sup>b</sup>Hormone receptor positive status was defined as either ER or PR positive.
Table 2. Associations between breast cancer HER2 and p95 content with DDFS in univariable survival analyses

<table>
<thead>
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<th>Treatment arm and variable</th>
<th>HR\textsuperscript{a}</th>
<th>P-value</th>
</tr>
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<tr>
<td>Chemotherapy Alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\log_{10}(\text{HER2})$</td>
<td>1.2</td>
<td>.30</td>
</tr>
<tr>
<td>$\log_{10}(\text{p95})$</td>
<td>2.0</td>
<td>.02</td>
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<tr>
<td>Chemotherapy + trastuzumab</td>
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<td></td>
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<tr>
<td>$\log_{10}(\text{HER2})$</td>
<td>0.98</td>
<td>.93</td>
</tr>
<tr>
<td>$\log_{10}(\text{p95})$</td>
<td>0.58</td>
<td>.19</td>
</tr>
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</table>

HER2, human epidermal growth factor receptor 2; HR, hazard ratio
Stratification was by cancer hormone receptor status, nodal status and chemotherapy type
\textsuperscript{a} Hazard ratios for 2-fold change in $\log_{10}(\text{HER2})$ or $\log_{10}(\text{p95})$
Table 3. Multivariable predictive models for DDFS

<table>
<thead>
<tr>
<th>Variable</th>
<th>HER2 model</th>
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<th>p95 model</th>
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<td></td>
<td>HR</td>
<td>P-value</td>
<td>HR</td>
<td>P-value</td>
</tr>
<tr>
<td>Node positive cancer</td>
<td>5.2</td>
<td>.03</td>
<td>6.3</td>
<td>.01</td>
</tr>
<tr>
<td>Vinorelbine-containing chemotherapy</td>
<td>1.8</td>
<td>.06</td>
<td>1.8</td>
<td>.07</td>
</tr>
<tr>
<td>ER and/or PR positive cancer</td>
<td>0.76</td>
<td>.38</td>
<td>0.76</td>
<td>.38</td>
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<tr>
<td>Trastuzumab administered</td>
<td>0.53</td>
<td>.04</td>
<td>0.55</td>
<td>.06</td>
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<tr>
<td>Log_{10}(HER2)\textsuperscript{a}</td>
<td>1.6</td>
<td>.27</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Log_{10}(HER2) : trastuzumab interaction\textsuperscript{a}</td>
<td>0.58</td>
<td>.35</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Log_{10}(p95)\textsuperscript{a}</td>
<td>NC</td>
<td>NC</td>
<td>5.0</td>
<td>.01</td>
</tr>
<tr>
<td>Log_{10}(p95) : trastuzumab interaction\textsuperscript{a}</td>
<td>NC</td>
<td>NC</td>
<td>0.06</td>
<td>.01</td>
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</tbody>
</table>

HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; NC, not considered.
\textsuperscript{a} Hazard ratios for 2-fold change in log\textsubscript{10}(HER2) or log\textsubscript{10}(p95)
Table 4. Multivariable predictive models of p95 for DDFS in subsets of patients defined by breast cancer steroid hormone receptor expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hormone receptor negative</th>
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<th>Hormone receptor positive</th>
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<tr>
<td></td>
<td>HR</td>
<td>P-value</td>
<td>HR</td>
<td>P-value</td>
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<tr>
<td>Node positive</td>
<td>-- (^a)</td>
<td>1</td>
<td>1.1</td>
<td>.93</td>
</tr>
<tr>
<td>Vinorelbine-containing chemotherapy</td>
<td>1.7</td>
<td>.26</td>
<td>1.8</td>
<td>.26</td>
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<tr>
<td>Trastuzumab administered</td>
<td>0.98</td>
<td>.98</td>
<td>0.47</td>
<td>.14</td>
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<tr>
<td>(\log_{10}(p95)) (^b)</td>
<td>19.7</td>
<td>.009</td>
<td>1.3</td>
<td>.81</td>
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<tr>
<td>(\log_{10}(p95)) : trastuzumab interaction (^b)</td>
<td>0.002</td>
<td>.009</td>
<td>0.79</td>
<td>.90</td>
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</table>

HR, hazard ratio

\(^a\) Estimate of hazard ratio could not be determined due to a low number of node positive patients

\(^b\) Hazard ratios for 2-fold change in \(\log_{10}(p95)\)
Figure 2

A. p95-negative

HR = 0.84  $P = .66$

B. p95-positive

HR = 0.32  $P = .03$

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