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Evaluation of the Role of p95 HER2 Isoform in Trastuzumab Efficacy in Metastatic Breast Cancer

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Abstract. *Background/Aim: Human epidermal growth factor receptor 2 (HER2) P95-isoform could be involved in trastuzumab resistance in HER2 metastatic breast cancer. Materials and Methods: A total of 114 metastatic breast cancer patients treated with trastuzumab were evaluated retrospectively. HER2 was centrally reviewed. P95 was evaluated along with other markers possibly affecting trastuzumab efficacy in regards to progression-free survival and overall survival. Results: HER2 was centrally negative in 54 cases. P95 expression was significantly higher in HER2-positive tumors. High p95 was associated with gain of HER2 copy number variations (CNVs), high pHER2Tyr877, Ki67 and HER2 mRNA. P95 as a continuous variable was positively correlated with mRNA expression of HER2 and negatively correlated with HER4 and IGF1. HER2-negative p95-high patients had a marginally higher risk for death (HR=2.15, p=0.055). Conclusion: p95 was associated with higher HER2 CNVs and mRNA expression, pHER2Tyr877 expression and high Ki67, indicating a more aggressive phenotype.*

Over-expression of the cell surface human epidermal growth factor receptor 2 (HER2) is observed in almost 20% of breast tumors usually due to *HER2* gene amplification and this has

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been related to worse prognosis and more frequent disease recurrence (1). The use of the monoclonal antibody, trastuzumab, that selectively targets the extracellular domain of the HER2 receptor, has led to improved response rates, time to disease progression and overall survival in metastatic breast cancer patients with HER2 over-expressing and/or amplified tumors (2). Primary resistance to treatment with trastuzumab is noted in many patients with HER2 positive tumors, while most of the responding patients develop resistance early in the course of treatment *i.e.* in the first year (3, 4). Detection of HER2 protein over-expression or *HER2* gene amplification is a validated predictive factor for trastuzumab efficacy, however, it is still unclear which patients with HER2 positive disease will not benefit from treatment.

Almost 30% of HER2 positive breast tumors express p95HER2, a truncated form of HER2, which has no extracellular domain. p95HER2 fragments usually arise from proteolytic cleavage of the extracellular domain of the receptor or from internal *HER2* mRNA initiation codons (5, 6). These fragments are oncogenic despite lacking most of the extracellular domain. They form homodimers by intermolecular disulfide bonds, drive breast cancer progression *in vivo* by activating multiple tumor growth and metastasis signaling cascades, and are related to a more aggressive phenotype than full length HER2 (6). The expression of p95HER2 in breast cancer has been associated with higher incidence of metastases to lymph nodes (7) and worse prognosis in HER2 positive patients (8). Additionally, it has been associated with resistance to trastuzumab treatment most likely due to the lack of a binding site for the antibody (9-11).

Many other factors have been involved in trastuzumab resistance. The estrogen receptor (ER) and progesterone receptor (PR) status has well-studied prognostic and predictive significance in all breast cancer patient groups and furthermore, crosstalk between ER and HER2 pathways seems to confer resistance in treatment including trastuzumab (12). Activation of the PI3K/Akt/mTOR pathway through oncogenic mutations of *PIK3CA* and loss of PTEN, have both been associated with trastuzumab resistance in breast tumors (13). Furthermore, expression of the p85 subunit of PI3K has been associated with shorter survival in HER2-positive breast cancer (14). The IGF-R pathway has also been linked to trastuzumab resistance, with higher expression of the pathway growth factor GLP-1 and the effector protein Akt negatively affecting outcome in trastuzumab-treated patients (15, 16). Src kinase is a common part of the IFG and HER pathways and its activation is linked to trastuzumab resistance (17, 18). Trastuzumab efficacy may also be affected by the status of other proteins of the HER family; *EGFR* gene copy gain adversely affects PFS in trastuzumab-treated patients, whereas higher expression of HER3 and HER4 may be beneficial (19, 20). Self-activation of HER2 protein by phosphorylation in tyrosine residues may also variably affect the results of trastuzumab use in breast cancer patients (21-23). The potential beneficial role of topoisomerase IIa over-expression in trastuzumab efficacy has also been under investigation, but its effect may be confounded by the frequent anthracycline use in these populations (24-26).

In this study, samples from patients with presumed HER2-positive metastatic breast cancer were analyzed with data extracted from the available medical records of patients treated with trastuzumab. The study included patients diagnosed in the early years of trastuzumab use when standardized diagnostic criteria for HER2 status were not available, thus HER2 status was centrally re-evaluated for all patients before studying p95 status and its correlation with other markers and patient outcome.

Materials and Methods

Eligibility criteria for this study included: a) histologically confirmed advanced (*de novo* metastatic or recurrent) breast cancer, b) available p95 data c) adequate formalin-fixed paraffin-embedded (FFPE) tumor tissue for evaluation of biological markers, d) treatment with trastuzumab for metastatic breast cancer and e) availability of data regarding patient and tumor characteristics, details of administered treatment, and outcome. Approval of the translational research protocol was obtained by the Bioethics Committee of the Aristotle University of Thessaloniki School of Medicine (4283; Jan 14 2008) under the general title "Investigation of major mechanisms of resistance to treatment with trastuzumab in patients with metastatic breast cancer". The medical records of a previously published cohort of metastatic breast cancer patients treated with a trastuzumab-based regimen (13, 27) enriched with new cases, were retrospectively reviewed.

Informed consent was obtained before receiving any treatment for all patients included in the study after 2005, for the provision of biological material for future research studies, while waiver of consent from the Committee was available for patients included in the study before 2005.

FFPE tumor tissue samples from 114 patients meeting the above eligibility criteria who were thought to carry HER2-positive tumors according to locally performed immunohistochemistry (IHC), were retrospectively collected. Central re-evaluation for HER2 status by fluorescent in situ hybridization (FISH), or by IHC (if FISH was not feasible) was performed for all samples along with ER, PR and Ki67 status assessment by IHC.

Data from earlier publications of our group were used for associations with the present findings. The methods for assessing all other biomarkers besides p95 have been previously described in detail as following: *PI3K* mutations SNP genotyping, PTEN IHC and FISH (13), p85 PI3k subunit IHC (14), *IGF1* mRNA, *GLP1-R* mRNA, *Akt1, Akt2, Akt3* mRNA, and mTor expression (16), *Src* mRNA (17), EGFR mRNA, CNVs and IHC, *HER2* mRNA and CNVs, *HER3* mRNA, IHC and CNVs, HER4 mRNA and IHC, HER pTyr1221/1222 and pTyr877 (19), topoisomerase IIa IHC (24).

For the purpose of this study, p95 was evaluated with the VeraTag p95 assay that uses a proprietary p95 monoclonal antibody (D9, Monogram Biosciences) and has been described in detail in the publication that established the method (10). In summary, D9

specifically recognizes the highly active M611-HER2-CTF form of truncated HER2, but is sterically blocked from binding to full-length HER2. D9 binding to the FFPE tumor section was detected by a secondary antibody conjugated to a fluorescent VeraTag reporter molecule. The VeraTag reporter was released by reduction and quantified by capillary electrophoresis. Multiple cell line controls were included in each batch for normalization. Relative fluorescence (RF) was normalized to tumor area to give units of RF/mm^2. The prespecified p95 2.8 RF/mm^2 cutoff, derived from an independent training set, as previously described (10), has been validated in an independent cohort of HER2-positive metastatic breast cancer patients treated with trastuzumab regarding PFS and OS (28).

Statistical considerations. Basic clinicopathological parameters of interest and p95 expression for the total cohort and by HER2 status (according to central evaluation) were summarized using descriptive statistical methods. Comparisons between p95 and other markers of interest were evaluated with the chi-square (or Fisher's exact if more appropriate) and the Wilcoxon rank-sum tests. Spearman correlations were used to assess the association of continuous variables.

Endpoints included 1) progression-free survival (PFS) defined as the time from the first day of trastuzumab treatment (with or without simultaneous administration of chemotherapy/hormonal therapy) to the day of the first disease progression, death (due to any cause) or last contact (whichever occurred first), 2) overall survival (OS) calculated from the date of breast cancer diagnosis until death from any cause or last contact and 3) survival measured from the first day of trastuzumab treatment for metastatic breast cancer until death or last contact. The Kaplan–Meier method was used for the estimation of survival functions. The log-rank test was applied for comparison of survival curves.

The effect of p95 expression on PFS, OS and survival was evaluated using 2.8 as the cut-off value to categorize tumors into two groups [low (p95<2.8) *vs*. high (p95≥2.8)] for comparisons (10, 28). Cox regression models were applied to estimate the risk of progression/death according to p95 expression. The proportionality assessment was tested using time-dependent covariates.
The study were conducted in line with the state-of-the-art guidelines for tumor marker prognostic studies (29).

Updated follow-up data for all patients were obtained in October 2019. All applied tests were two-sided and significance was set at 5%. The SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) software was used for statistical analysis.

Results

Patient and tumor characteristics. Overall, 114 women treated with trastuzumab for advanced breast cancer between 09/1999 and 04/2012 with available data for p95 were included in the analysis. According to the local assessment, all patients had HER2-positive breast cancer and had therefore been treated with trastuzumab-based regimens. However, upon central re-evaluation of all tumors for HER2, it was found that 54 patients (47.4%) had truly HER2-negative tumors.

Clinical and tumor characteristics were well balanced between patients with HER2-positive and HER2-negative disease according to the central HER2 evaluation (Table I). In total, 36 patients (31.6%) had metastatic disease at the time of the initial diagnosis (*de novo* metastatic breast cancer), whereas the rest of the patients (68.4%) were diagnosed with early-stage breast cancer (R-metastatic breast cancer). The median age at the time of trastuzumab administration was 57 years (range=29-95 years) and most patients were postmenopausal (74.6%) with PS 0 (77.2%) and higher-grade tumors.

Treatment details. Overall, 100 patients (45 with HER2-negative and 55 with HER2-positive disease) were treated with first-line trastuzumab, while in 14 patients (9 HER2-negative and 5 HER2-positive) trastuzumab was administered as a second-line treatment. In most patients it was given with concurrent chemotherapy (45 HER2-negative; 57 HER2-positive), while in 10 patients (8 HER2-negative and 2 HER2-positive) it was administered along with hormonal therapy. In addition, 2 patients (one HER2-positive and one HER2-negative) received trastuzumab as a monotherapy. Of the 100 first-line trastuzumab treated patients, 90 had it combined with chemotherapy, 9 with hormonal therapy and 1 as a monotherapy. Among patients who were treated with second-line trastuzumab, the drug was administered with concurrent chemotherapy in 12 patients, hormonal therapy in 1 patient and as a monotherapy in one additional patient. Thirty-four patients (29.8%) received lapatinib following the first administration of trastuzumab for metastatic breast cancer.

P95 distribution and associations with other markers. The median p95 value in the entire cohort was 1.8, ranging from 0.5 to 11.8 and eighty-five patients (74.6%) had low p95 expression. The median p95 value among patients with HER2-positive tumors was 2.2 (range=0.5-8.5) and was significantly higher as opposed to those with HER2-negative tumors (median p95: 1.4, min-max: 0.5-11.8, Wilcoxon rank-sum $p<0.001$) (Figure 1).

The associations of p95 expression with selected markers of interest for the total cohort are presented in Table II. Patients carrying tumors with high p95 had more frequently negative ER/PR status (chi-square $p=0.029$), high gain of *HER2* CNVs (Fisher's $p<0.001$), positive pHER2Tyr^{877} protein expression (chi-square $p=0.043$) as well as higher Ki67 and *HER2* mRNA expression than patients with low p95 (Wilcoxon rank-sum $p=0.020$ and $p=0.028$, respectively). In addition, p95 was positively correlated as a continuous variable with *HER2* mRNA expression (spearman rho=0.40, $p<0.001$) and negatively correlated with *HER4* (rho=–0.34, $p=0.003$) and *IGF1* mRNA expression (rho=–0.29, $p=0.01$).

Association of p95 with clinical outcomes. Within a median follow-up time of 15.5 years since diagnosis (95%CI=13.6-

Table I. *Clinical and tumor characteristics by HER2 status (based on central assessment).*

	Total (N=114)	HER2-negative (N=54)	HER2-positive (N=60)
Age*			
Median (min, max)	56.9 (28.9, 95.0)	59.3 (32.7, 75.8)	54.6 (28.9, 95.0)
Menopausal status*			
Postmenopausal	85 (74.6)	40 (74.1)	45 (75.0)
Premenopausal	29 (25.4)	14 (25.9)	15 (25.0)
PS*			
0	88 (77.2)	41 (75.9)	47 (78.3)
1	20 (17.5)	10 (18.5)	10 (16.7)
2	5 (4.4)	2 (3.7)	3 (5.0)
Unknown	1 (0.88)	1 (1.9)	0 (0.0)
Histological grade			
I	1 (0.88)	0 (0.0)	1 (1.7)
II	39 (34.2)	18 (33.3)	21 (35.0)
III	67 (58.8)	31 (57.4)	36 (60.0)
Unknown	7 (6.1)	5 (9.3)	2 (3.3)
Subtypes			
Luminal A	9 (7.9)	9 (16.7)	0 (0.0)
Luminal B	32 (28.1)	32 (59.3)	0 (0.0)
Luminal HER2	38 (33.3)	0 (0.0)	38 (63.3)
HER2-enriched	22 (19.3)	0 (0.0)	22 (36.7)
Triple-negative	11 (9.6)	11 (20.4)	0 (0.0)
Unknown	2 (1.8)	2 (3.7)	0 (0.0)
No. of metastatic sites*			
1-2	104 (91.2)	48 (88.9)	56 (93.3)
≥3	9 (7.9)	5 (9.3)	4 (6.7)
Unknown	1 (0.88)	1 (1.9)	0 (0.0)
Visceral metastases*			
Yes	79 (69.3)	34 (63.0)	45 (75.0)
No	34 (29.8)	19 (35.2)	15 (25.0)
Unknown	1 (0.88)	1 (1.9)	0 (0.0)
De novo MBC	36 (31.6)	17 (31.5)	19 (31.7)
Biopsy only^	8 (22.2)	5 (29.4)	3 (15.8)
Modified radical^	13 (36.1)	4 (23.5)	9 (47.4)
Partial mastectomy^	10 (27.8)	6 (35.3)	4 (21.1)
Simple mastectomy^	5 (13.9)	2 (11.8)	3 (15.8)
R-MBC	78 (68.4)	37 (68.5)	41 (68.3)
Adjuvant CT^^	63 (80.7)	33 (89.2)	30 (73.2)
CMF-based adjuvant CT^^	34 (43.6)	18 (48.6)	16 (39.0)
Taxane-based adjuvant CT^^	20 (25.6)	8 (21.6)	12 (29.3)
Anthracycline-based adjuvant CT^^	45 (57.7)	19 (51.4)	26 (63.4)
Adjuvant HT^^	54 (69.2)	29 (78.4)	25 (61.0)
Adjuvant RT^^	39 (50.0)	22 (59.5)	17 (41.5)

*At the time of trastuzumab initiation. ^Only for *de novo* MBC patients. ^^ Only for patients with R-MBC. HER2: Human epidermal growth factor receptor 2; MBC: metastatic breast cancer; CT: chemotherapy; CMF: cyclophosphamide/methotrexate/5 fluorouracil; HT: hormonal therapy; MBC: metastatic breast cancer; RT: radiotherapy.

19.7), 97 deaths were reported; 48 of them in HER2-positive and 49 in HER2-negative patients. Disease progression since the first administration of trastuzumab for advanced disease was detected in 89.5% of patients (50 HER2-negative and 52 HER2-positive). The median OS in the entire cohort was 5.6 years (95%CI=4.5-6.3) and did not differ between patients with HER2-positive and HER2-negative disease [median OS=5.6 (95%CI=4.3-6.2) *vs*. 5.6 (95%CI=4.4-7.6), log-rank *p*=0.17]. The median PFS and survival for the entire cohort were 1.1 year (95%CI=0.8-1.5) and 3.1 years (95%CI=2.7-3.8), respectively. Patients carrying HER2-positive tumors as opposed to patients with HER2-negative disease had significantly longer PFS [median PFS 1.5 years (95%CI=1.0-2.0) *vs*. 0.8 years (95%CI=0.6-1.2), *p*=0.028, Figure 2A] and survival [median survival 3.5 years (95%CI=2.8-4.3) *vs*. 2.7 years (95%CI=2.1-3.5), *p*=0.020, Figure 2B]. No significant

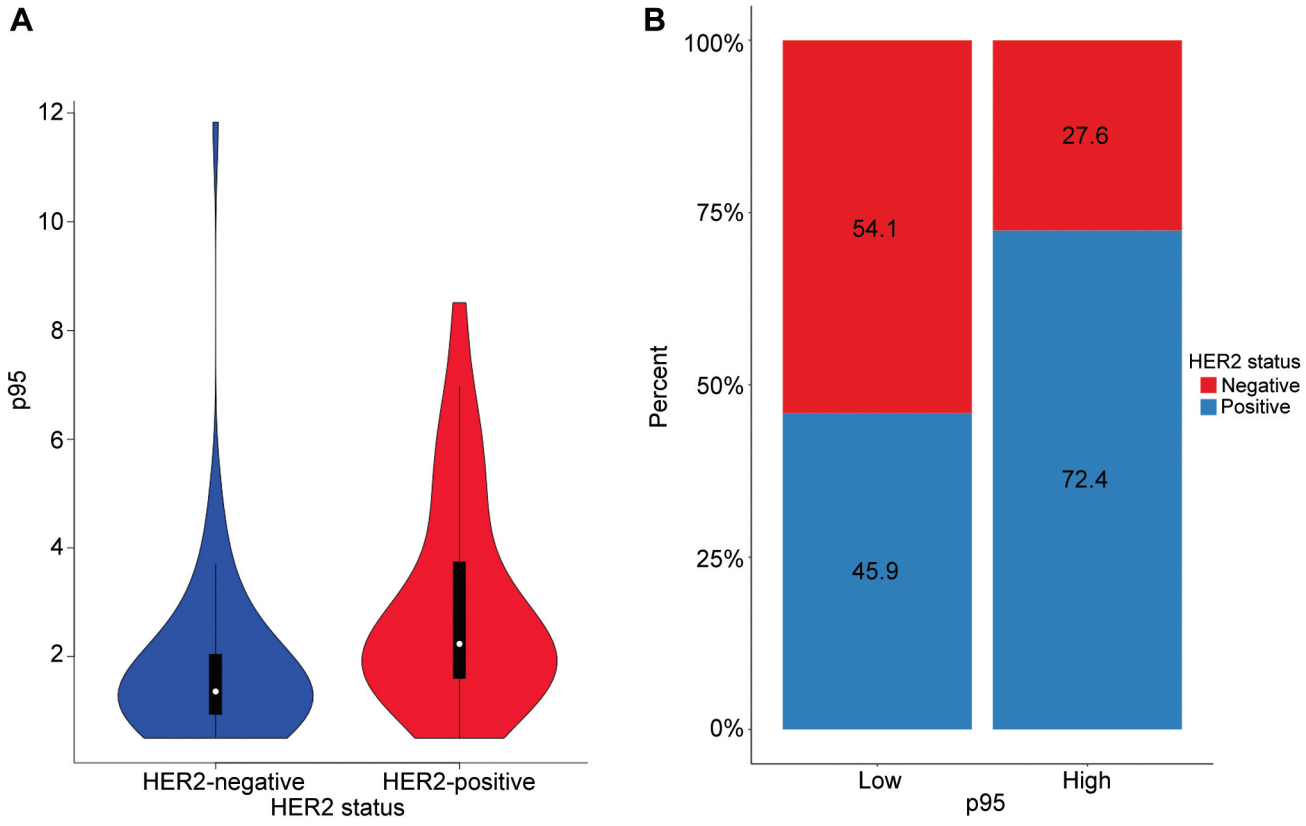

Figure 1. *Association of p95 expression with HER2 status. (A) Violin plot of p95 by HER2 status and (B) bar plot of p95 expression according to HER2 status.*

difference was found according to disease presentation status in terms of OS (p=0.20), PFS (p=0.39) or survival (p=0.22).

The prognostic significance of p95 was assessed separately among patients with HER2-positive and HER2-negative disease for OS, PFS and survival. P95 did not show prognostic significance for PFS and survival either among patients with HER2-positive or among those with HER2-negative disease (Table III). In terms of OS, patients with HER2-negative tumors and high p95 had marginally significantly increased risk of death as compared to those with low p95 (HR=2.15, 95%CI=0.98-4.70, Wald's p=0.055, Figure 3). It is of note, however, that only 8 of the 54 HER2-negative women had low p95 expression and all of them had died at the last follow-up. Because of the small sample size of our study and the limited number of patients with HER2-negative tumors and low p95 expression, multivariate analysis was not performed to assess the effect of p95 upon adjustment for significant clinicopathological parameters in this subgroup of the study population. Therefore, the aforementioned significant result in the HER2-negative subgroup needs to be interpreted with caution until validation in bigger cohorts.

Discussion

Our study evaluates the role of the p95 truncated isoform of HER2 receptor in trastuzumab-treated breast cancer patients, that has been associated with a more aggressive phenotype (6-8) with possibly intrinsic resistance to trastuzumab treatment (9, 10). Of note, there are conflicting data in the literature as other studies have not shown a negative predictive role for p95HER2 in trastuzumab treatment (30).

In our population, p95HER2 was positively associated with other markers indicative of a more aggressive phenotype. Higher Ki67 is a marker of increased cell proliferation, while regarding prognosis gain of *HER2* CNVs and higher *HER2* mRNA expression mark the amplification of HER2 expression and are associated with high- risk breast cancer disease (19). Additionally, higher *HER2* mRNA expression is also linked to increased trastuzumab response in metastatic breast cancer (31). The positive correlation of p95 with the higher incidence of detection of the phosphorylated activated form of HER2 (pHER2Tyr^{877}) could also link p95 to a more aggressive phenotype, as

Table II. *Associations of p95 with other markers in the entire cohort of patients*.

		P95		
	Total (N=114)	Low (N=85)	High (N=29)	*p*-Value
HER2 status (by central assessment)				0.013[c]
Negative	54 (47.4)	46 (54.1)	8 (27.6)	
Positive	60 (52.6)	39 (45.9)	21 (72.4)	
Ki67 (%)*	40.0 (1.00, 90.0)	40.0 (1.00, 90.0)	50.0 (10.0, 80.0)	**0.020**[b]
Src mRNA*	40.3 (36.0, 43.7)	40.3 (36.0, 43.7)	40.2 (37.7, 42.5)	0.99[b]
AKT1 mRNA*	42.4 (38.3, 44.8)	42.3 (38.3, 44.8)	42.4 (39.8, 43.7)	0.63[b]
AKT2 mRNA*	36.4 (27.0, 38.7)	36.5 (27.0, 38.7)	36.2 (28.7, 38.6)	0.50[b]
AKT3 mRNA*	36.1 (27.0, 39.4)	36.1 (27.0, 39.4)	36.1 (27.2, 37.9)	0.25[b]
GLP1-R mRNA*	28.8 (23.7, 36.3)	28.8 (25.9, 36.3)	28.5 (23.7, 30.6)	0.26[b]
IGF1 mRNA*	35.7 (28.3, 40.4)	36.0 (28.3, 39.4)	34.9 (31.0, 40.4)	0.088[b]
EGFR mRNA*	37.3 (28.3, 40.6)	37.4 (28.7, 40.6)	36.7 (28.3, 40.1)	0.18[b]
HER2 mRNA*	39.6 (27.0, 44.4)	39.3 (27.0, 42.9)	41.0 (36.3, 44.4)	**0.028**[b]
HER3 mRNA*	41.0 (38.3, 43.7)	41.2 (38.3, 43.7)	40.6 (38.8, 43.0)	0.32[b]
HER4 mRNA *	36.4 (26.9, 40.4)	36.8 (26.9, 40.4)	35.2 (27.2, 39.8)	0.11[b]
EGFR CNVs *				0.24[c]
No gain	85 (90.4)	60 (88.2)	25 (96.2)	
Gain	9 (9.6)	8 (11.8)	1 (3.8)	
HER2 CNVs*				**<0.001**[c]
No gain	40 (40.0)	32 (43.8)	8 (29.6)	
Low gain	36 (36.0)	32 (43.8)	4 (14.8)	
High gain	24 (24.0)	9 (12.3)	15 (55.6)	
HER3 CNVs*				0.76[c]
No gain	81 (90.0)	58 (90.6)	23 (88.5)	
Gain	9 (10.0)	6 (9.4)	3 (11.5)	
EGFR protein expression*				0.086[c]
Negative	84 (84.8)	63 (88.7)	21 (75.0)	
Positive	15 (15.2)	8 (11.3)	7 (25.0)	
pHER2Tyr$^{1221/1222}$ protein expression*				0.28[c]
Negative	68 (68.7)	51 (71.8)	17 (60.7)	
Positive	31 (31.3)	20 (28.2)	11 (39.3)	
pHER2Tyr^{877} protein expression*				**0.043**[c]
Negative	79 (80.6)	60 (85.7)	19 (67.9)	
Positive	19 (19.4)	10 (14.3)	9 (32.1)	
HER3 protein expression*				0.38[c]
Negative	25 (27.8)	20 (30.3)	5 (20.8)	
Positive	65 (72.2)	46 (69.7)	19 (79.2)	
HER4 protein expression*				0.12[c]
Negative	12 (13.3)	11 (16.7)	1 (4.2)	
Positive	78 (86.7)	55 (83.3)	23 (95.8)	
P85 expression protein expression*				0.69[c]
Negative	37 (34.9)	26 (33.8)	11 (37.9)	
Positive	69 (65.1)	51 (66.2)	18 (62.1)	
PIK3CA status*				**0.048**[c]
Wild-type	80 (83.3)	56 (78.9)	24 (96.0)	
Mutated	16 (16.7)	15 (21.1)	1 (4.0)	
ER/PgR status				**0.029**[c]
Negative	33 (28.9)	20 (23.5)	13 (44.8)	
Positive	81 (71.1)	65 (76.5)	16 (55.2)	
PTEN status*				0.11[c]
Loss	68 (62.4)	47 (58.0)	21 (75.0)	
No loss	41 (37.6)	34 (42.0)	7 (25.0)	
mTOR protein expression*				0.38[c]
Negative	40 (37.7)	31 (40.3)	9 (31.0)	
Positive	66 (62.3)	46 (59.7)	20 (69.0)	
TOPOIIa protein expression*				0.80[c]
Negative	42 (42.4)	30 (41.7)	12 (44.4)	
Positive	57 (57.6)	42 (58.3)	15 (55.6)	

*Missing data: Ki67 (%)=3, *Src* mRNA=16, *AKT1* mRNA=41, *AKT2* mRNA=46, *AKT3* mRNA=45, *GLP1-R* mRNA=42, *IGF1* mRNA=36, *EGFR* mRNA=44, *HER2* mRNA=41, *HER3* mRNA=44, *HER4* mRNA=43, *EGFR* CNVs=20, *HER3* CNVs=24, *HER2* CNVs=14, *EGFR* protein expression=15, pHER2Tyr$^{1221/1222}$ protein expression=15, pHER2Tyr^{877} protein expression=16, HER3 protein expression=24, HER4 protein expression=24, P85 protein expression=8, *PI3KCA* mutation=18, PTEN status=5, mTOR protein expression=8, TOPOIIa protein expression=15. Values summarized as Median with minimum, maximum or Number with column percent. *p*-Values: [b]Wilcoxon rank-sum; [c]Pearson's chi-square/Fisher's exact. Significant *p*-Values are shown in bold.

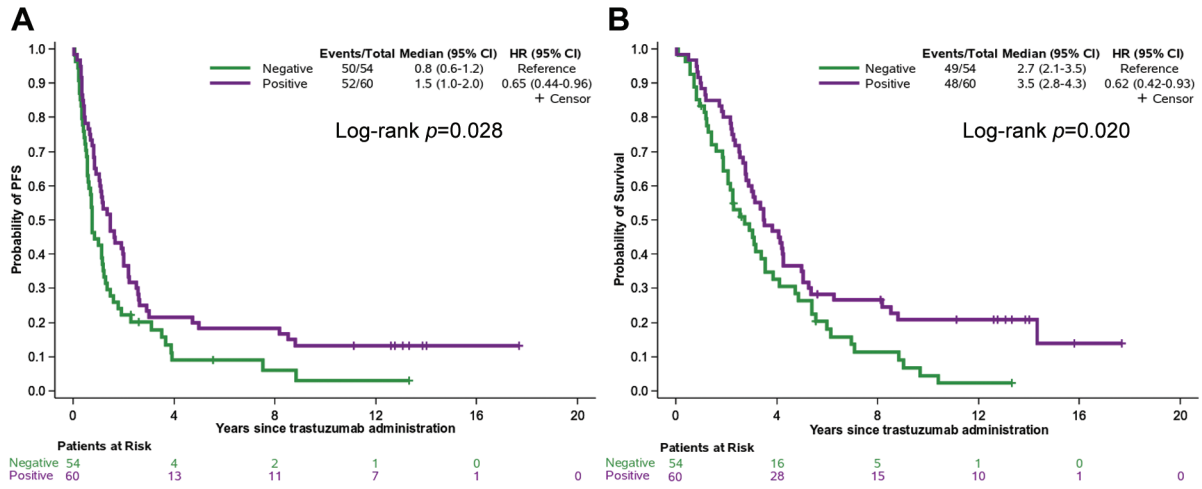

Figure 2. *Kaplan–Meier curves based on HER2 status for (A) progression-free survival (PFS) and (B) survival.*

studies have shown that the phosphorylation of HER2 can confer resistance to trastuzumab treatment (23, 32).

HER4 carries a positive prognostic value (33) and the negative correlation of *HER4* mRNA expression as a continuous variable with p95 expression is in accordance with p95 marking a more aggressive disease. Of note, IGF-1 pathway is a crossroad of convergence for many signaling pathways implicated in breast cancer growth and it seems that its activation confers resistance to trastuzumab (16). The negative correlation of *IGF1* mRNA expression with p95 could indicate that these two pathways of resistance in trastuzumab-treated patients are not activated at the same time.

Of interest, p95HER2 positivity did not seem to have a significant effect on the PFS and OS of the HER2- positive trastuzumab-treated breast cancer patients in our study. It is worth mentioning that our study has a group of trastuzumab-treated HER2-negative patients in which survival was shorter than that of HER2-positive patients. This fact highlights how effective trastuzumab is in HER2-positive patients, who prior to the trastuzumab era had an aggressive course of disease with very bad prognosis.

Interestingly, in the HER2-negative subgroup overall survival was marginally significantly lower in patients with high p95 expression. Although the small number of patients makes this finding of uncertain importance, this signal is consistent with the independent negative prognostic role of p95HER2, even in the absence of HER2 positivity.

Regarding treatment options in p95HER2 positive patients, the TKI inhibitor lapatinib has been of interest as preclinical studies have shown equal effects in p95HER2 positive and negative patients (34). The lack of an extracellular domain in the p95HER2 variant that precludes trastuzumab binding does not affect the intracellular lapatinib binding spot, thus it is not a surprise that lapatinib is active in p95HER2 patients (34). Thirty-four patients (29.8%) received lapatinib following trastuzumab in our group of metastatic breast cancer patients, but we have no data on its efficacy in respect to p95 status.

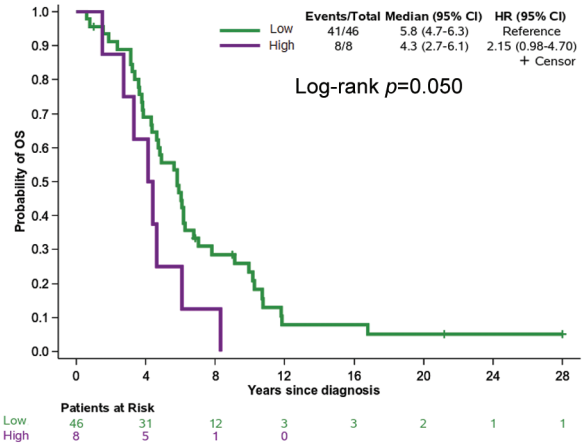

Figure 3. *Kaplan–Meier curves based on p95 expression for overall survival (OS) in patients with HER2-negative tumors.*

Our study has the disadvantages of all retrospective analyses, especially those that come from a non-trial population. As trastuzumab resistance remains a clinical problem, further prospective studies that include translational research are warranted to discover more accurate predictive factors for better patient selection. Additionally, the outcome of patients with low *vs*. high p95 on trastuzumab, *i.e*. response rates and relapse-free interval, could be investigated prospectively.

Table III. *Results of univariate Cox regression models for OS, PFS and survival based on p95 expression.*

	HER2-positive (N=60)				HER2-negative (N=54)			
P95	Event/Total	Median* (95%CI)	HR (95%CI)	*p*-Value	Event/Total	Median* (95%CI)	HR (95%CI)	*p*-Value
OS								
Low	29/39	7.0 (4.41-8.47)	Reference	--	41/46	5.83 (4.35-6.27)	Reference	--
High	19/21	4.52 (3.39-6.59)	1.63 (0.91-2.92)	0.10	8/8	4.26 (1.51-6.10)	2.15 (0.98-4.70)	0.055
PFS								
Low	32/39	1.66 (0.80-2.24)	Reference	--	42/46	0.77 (0.57-1.24)	Reference	--
High	20/21	1.14 (0.85-2.01)	1.37 (0.78-2.41)	0.27	8/8	0.56 (0.26-1.59)	1.62 (0.75-3.49)	0.22
Survival								
Low	29/39	4.14 (2.66-5.35)	Reference	--	41/46	2.92 (1.88-4.10)	Reference	--
High	19/21	3.49 (2.29-4.20)	1.48 (0.82-2.67)	0.19	8/8	2.42 (1.30-3.54)	1.29 (0.60-2.78)	0.52

HR: Hazard ratio; CI: confidence interval; OS: overall survival; PFS: progression-free survival. *in years.

In conclusion, our study did not demonstrate a significant predictive role for p95HER2 in trastuzumab efficacy in metastatic breast cancer patients, but it is in line with previous studies associating p95HER2 with negative prognostic factors and a more aggressive phenotype.

Conflicts of Interest

ER: Consulting or Advisory Role: AstraZeneca, Bristol-Myers Squibb, Pfizer, Research Funding: Novartis, Demo Pharmaceutical, Celldex, Radius Health, Tesaro, Parexel, Anabiosis Pharmaceuticals. PP: Advisory Role: Roche, Merck, Genesis Pharmaceuticals, Honoraria: Roche, Merck; AK: Consulting or advisory role: Amgen, Roche, BMS, Astra-Zeneca, MSD, Ipsen; DP: Advisory Role: Roche, MSD, Astellas. Honoraria: Roche, MSD, Astellas; A.K. Advisory role: Novartis, Roche, Genesis, Astra-Zeneca, Speaker's bureau: GSK, Travel Accommodations: Genesis, Sanofi-Aventis, Amgen, BMS, Merck Serono; C.C. Advisory Role: Merck, Genesis Pharmaceuticals, Pfizer, Novartis, Roche, AstraZeneca, Bristol Myers Squibb. Honoraria: Roche, Bristol Myers Squibb. Travel: AZ, Sanofi; G.F. Advisory Board of Pfizer, Novartis and Roche. Honoraria from Astra-Zeneca. Genprex, Daiichi Sankyo, Ariad, RFL Holdings, Formycon stock ownership.

Authors' Contributions

Conceptualization: GR, ER, JS; Formal analysis: GAK; Investigation: ET, JS, SC; Resources: GR, ER, GO, GZ, EP, AB, HPK, PP, DB, NIA, ER, AK, DP, AK, CC, GF; Supervision: ER, GF; Writing – original draft: GR, ER, GAK, GF. Writing – review and editing: all Authors.

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