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ORIGINAL ARTICLE

PTEN loss is a predictive marker for HER2-positive metastatic breast cancer patients treated with trastuzumab-based therapies

Kemal Tekesin[1], Emre Akar[2], Mehmet Emin Gunes[3], Savas Bayrak[3], Tulin Ozturk[4], Serdar Altinay[5], Deniz Tural[2]

[1]Department of Surgery, Umraniye Training and Research Hospital, Istanbul, Turkey; [2]Department of Medical Oncology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey; [3]Department of Surgery, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey; [4]Department of Pathology, Cerrahpasa School of Medicine, Istanbul, Turkey; [5]Department of Pathology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey.

Summary

Purpose: *The purpose of this study was to retrospectively investigate the response to trastuzumab in breast cancer patients in terms of the potential roles of several oncogenic pathways (phosphatase and tensin homolog (PTEN) and phosphatidylinositol 3-kinase (PI3K)) in relation to HER2 status.*

Methods: *Paraffin-embedded primary tumor tissues of 100 HER2 positive metastatic breast cancer patients who received trastuzumab were analyzed with immunohistochemistry for p85 (PI3K) and PTEN. The relationship between variables was tested via chi-square, Fischer's exact test and Mann-Whitney U test, where appropriate. Progression-free survival (PFS) and overall survival (OS) were calculated with the Kaplan-Meier method and survival curves of subgroups were compared with the log-rank test.*

Results: *The level of immunohistochemical expression of PI3K was 42%. Loss of PTEN was observed in 43% of the patients. PTEN-expressing tumors had statistically higher response rates for trastuzumab than tumors not-expressing PTEN (p=0.012). PI3K expression had no significant effect on trastuzumab response. Median PFS for PTEN-expressing and not-expressing tumors were 15.3 months (95% CI, 12.6-34) and 12.1 months (95% CI, 7.9-16.2), respectively (p=0.04). The level of PI3K expression had no effect on PFS and OS in our patient population.*

Conclusions: *Loss of PTEN predicted poorer response to trastuzumab treatment and shorter PFS but not OS. We could not find an effect of PI3K expression on the above-mentioned parameters.*

Key words: *PTEN, PI3K, trastuzumab, breast cancer*

Introduction

Amplified HER2 protooncogene has proved to be an extremely important target in the management of breast cancer [1]. Trastuzumab has been the mainstay of treatment, both for early and advanced stage disease and newer agents that have been approved have changed the landscape of HER2-positive breast cancer management in an unprecedented way [2-4]. Despite all these important strides, more than half of the patients are resistant at the start or acquire resistance within a year or so [5,6]. The mechanisms of resistance are complex and currently no predictive biomarkers are available to guide treatment. Biomarkers of potential predictive value that might be associated with trastuzumab response and outcome include the PTEN mutations and deranged PI3K pathway.

Corresponding author: Deniz Tural, MD. Department of Medical Oncology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey.
Tel: +90 212 414 71 71, Fax: +90 212 414 71 72, Email: deniztural@gmail.com
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In this study, we retrospectively analyzed these three parameters on archival material of patients with HER2-positive advanced breast cancer who had been treated with trastuzumab as first-line treatment and tried to correlate their expression in relation to trastuzumab response and outcome.

Methods

The study protocol was approved by the Ethics Committee of Bakirkoy Dr. Sadi Konuk Training and Research hospital.

Inclusion criteria

One hundred patients with HER2-positive metastatic breast cancer and who received first-line trastuzumab-based therapies were included. Informed consent was obtained from the patients or the patient's next of kin.

Primary tumor tissues were analyzed for PI3K and PTEN by immunohistochemistry (IHC). HER2-positive patients who relapsed within one year of completion of adjuvant treatment were excluded.

HER2 analysis

HER2 status was analyzed using Novacastra, clone CB11 antibody at a 1/80 dilution.

IHC 1+ was characterized as 'negative' and 3+ as 'positive'. IHC 2+ cases were further analyzed by FISH or SISH at a Ventana Benchmark XT or LT. Amplification was defined as greater than 2.2 HER2/CEP 17 ratio [7].

Immunohistochemical staining of PTEN and anti-PI3K (p85) antibody

Paraffin-embedded blocks of primary breast cancer tissue specimens were cut into 5 μm thick sections. One section from each block was stained with hematoxylin and eosin as a control. Antigen retrieval was carried out by EDTA following deparaffinization.

Rabbit p27 monoclonal antibody for PTEN (28H6, Gene Tex, GTX73862, 1B4 Novacastra, NCL-P27, 1/20 dilution) and rabbit polyclonal to PI3 Kinase p85 alpha + Gamma antibody (Abcam Inc., 1 Kendall Square, Suite B2304 Cambridge, MA 02139-1517 USA. Cat no: ab74136, lot no: 835-834, 1/50 dilution) were utilized for PI3K determination.

Evaluation of PTEN and PI3K immunohistochemical staining

PTEN staining was mainly cytoplasmic. Levels of PTEN expression were scored semiquantitatively based on staining intensity and distribution using immunoreactive score (IRS) as described previoussly [8]. Intensity was scored according to a four-tier system: 0, no staining; 1, weak; 2, moderate; and 3, strong. We attributed one, two, three or four additional points if the percentage of positive cells was less than <5%, 5%-25% to 26%-0%, 51%-75 or greater than 75%, respectively. Specimens were defined as positive if the score was 7 or greater.

Based on quantitative evolution, tumors were considered positive for PI3K when 50% of the neoplastic cell showed distinct cytoplasmic staining [9].

The results of PTEN and PI3K immunohistochemical staining are shown in Figure 1.

Trastuzumab administration

Trastuzumab was administered as 6 mg/kg every 3 weeks for 52 weeks after a 8 mg/kg loading dose.

Treatment evaluation

Treatment response was evaluated by computed tomography every 12 weeks after the initiation of trastuzumab-based chemotherapy, or whenever clinically needed. The Response Evaluation Criteria in Solid Tumors were used to classify tumor responses. For analysis, complete response (CR) and partial response (PR) rates were classified as objective response. Progression-free survival (PFS) was defined from the date of starting treatment with trastuzumab plus chemotherapy to disease progression. Overall survival (OS) was calculated from the date of initiation of trastuzumab plus chemotherapy to death due to any cause or loss to follow-up.

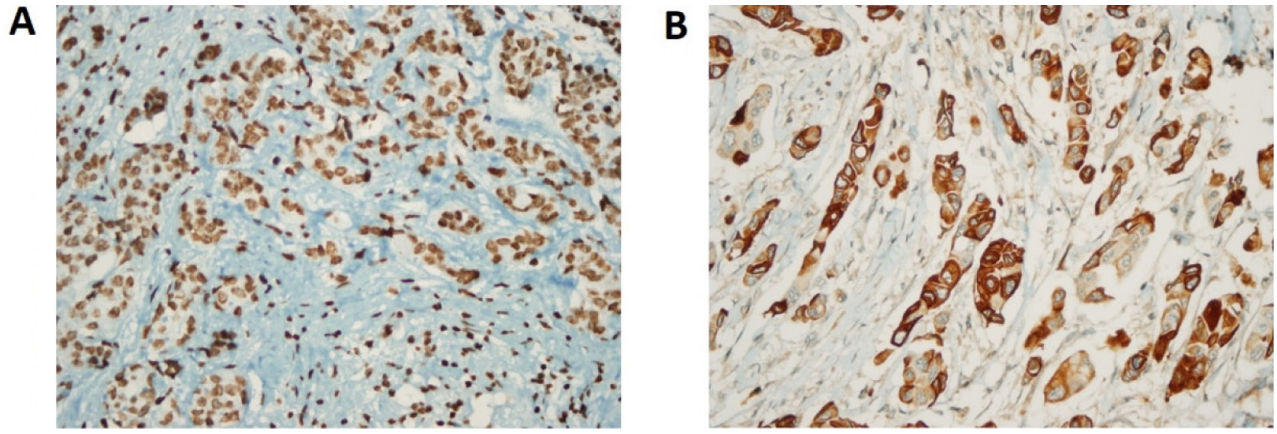

Figure 1. A: PTEN positive; Immunoreactive score (IRS) specimens were defined as positive when the score was 7 or greater. **B:** PI3K positive; Tumors were scored when 50% of the neoplastic cells showed a distinct cytoplasmic staining.

Statistics

Statistical analysis was done utilizing SPSS Version 15. Variables were compared by chi-square, Fischer's exact and Mann-Whitney U tests. RECIST criteria were used for response analysis. Kaplan-Meier curves were drawn to evaluate PFS and OS and the log-rank test was used to evaluate differences between groups. All variables that were significant in univariate analysis entered into a multivariate analysis. In backward, stepwise fashion, the significant univariate variable with the least significance was eliminated from the multivariate model. This was continued until only significant variables remained. The Cox proportional hazards model was used to calculate hazard ratio (HR) and 95% confidence intervals (95% CI). A p value<0.05 was considered significant.

Results

Clinicopathologic characteristics

One hundred eligible patients participated in the study. The median age of all patients was 55 years (range 28-76) with 40% being premenopausal. ERBB2 positivity (3+ staining by IHC) was found in 94% of the patients. All patients had invasive ductal carcinoma, only two had mixed lobular and ductal carcinoma. Fifty-eight percent (n=58) had grade III, 40% (n=40) grade II and only two patients (2%) had grade I tumor. Hormone receptors were positive in 59 patients. Sixty-four patients had visceral disease. Demographic characteristics are summarized in Table 1.

Treatment

Along with trastuzumab, first-line chemotherapy for metastatic disease included taxanes (82%), capecitabine (2%), vinorelbine (1%), and aromatase inhibitors as hormonal therapy in 15 patients. Five patients achieved CR, 48 had a PR, 24 had stable disease (SD), while 23 progressed despite therapy (PD).

Immunohistochemistry

All patients had adequate tumor tissue for immunohistochemical analysis. Fifty-seven patients were found to be positive for PTEN (57%). Accordingly, 58 patients were deemed to be PI3K-negative and 42 patients were PI3K-positive (42%).

The relation of PTEN and PI3K expression and response to trastuzumab treatment

Those patients with PR or CR to trastuzumab were defined as 'responders'. Response to trastuzumab was investigated in terms of PTEN status. Those with PTEN positive expression showed 28% PR, with 5% CR, while the PTEN-negative group had 20% PR with no CR. This difference was again statistically significant (p=0.012). In those with positive PI3K expression, response to trastuzumab was CR 2% and PR 22%, while CR 3% and PR 26% were noted in those with negative PI3K expression. This difference was not statistically significant (p=0.12).

Progression-free survival, PTEN, and PI3K status

The median PFS was 13 months (95% CI,10.1-16) in the whole group of patients who received trastuzumab and 15.3 months (95% CI,12.6-34) in PTEN-positive expressors versus 12.1 months (95% CI,7.9-16.2) in non-expressors (p=0.04) (Figure 2A) and 12.8 months (95% CI,9.2-16) in the group of patients expressing PI3K vs 14.3 months (95% CI,8.6-17.1) in patients with no PI3K expression (p=0.2) (Figure 2B).

Table 1. Demographic characteristics of 100 patients with HER2 positive breast cancer

Characteristics	*N*	*%*
Age (years), median (range)	55 (22-82)	
ECOG performance status		
0	20	20
1	75	75
2	5	5
Menopausal status		
Premenopausal	40	40
Postmenopausal	60	60
Hormone receptor status		
ER(+) or PR(+),	59	59
ER(+) and PR(+)	41	41
ER positive PR negative		
HER2 status		
HER2 IHC 3+	94	94
HER2 IHC 2+, FISH +	6	6
Number of metastatic sites		
1	54	54
2	26	26
≥3	20	20
Metastatic site		
Visceral organs	64	64
No visceral organs	36	36
Histology		
Invasive ductal	98	98
Invasive ductal+lobular	2	2
Tumor grade		
1	2	2
2	40	40
3	58	58

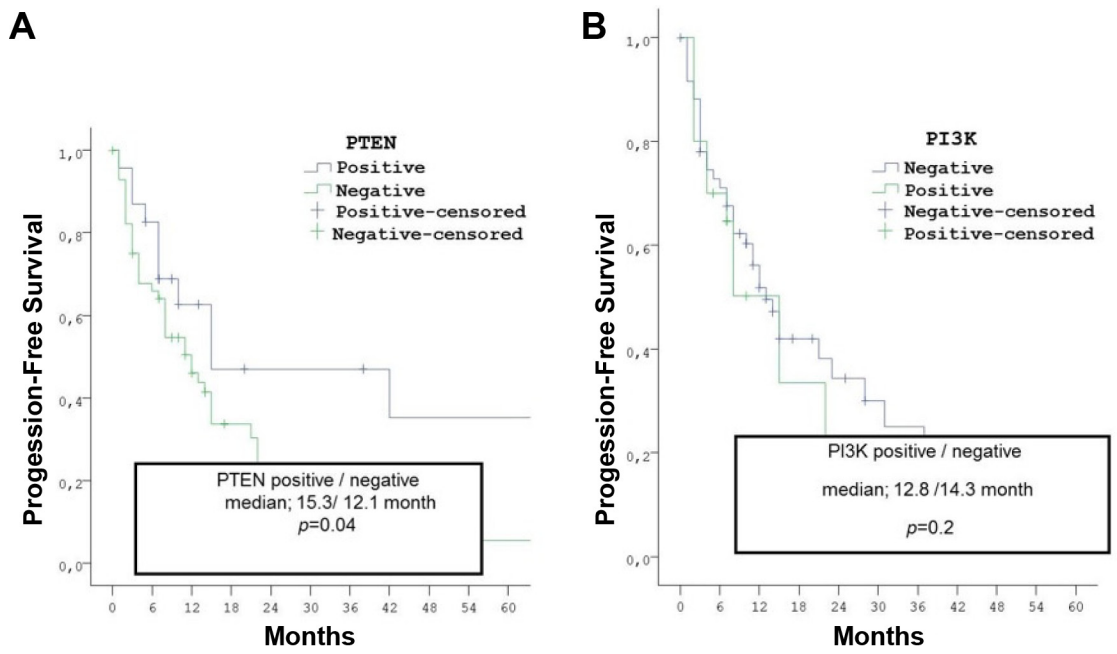

Figure 2. Patients' progression-free survival according to PTEN **(A)** and PI3K **(B)** statuses in HER2 positive metastatic breast cancer.

Table 2. Independent factors influencing PFS by Cox regression analysis

Factors	*p*	*HR (95% CI)*
Visceral metastases	0.009	
Present		2.38 (1.2-4.5)
Absent		1
Trastuzumab treatment response	0.014	
Absent		2.2 (1.18-4.47)
Present		1

Factors influencing progression-free survival by univariate analysis

Median PFS was significantly prolonged in patients responding to trastuzumab and with no visceral metastases. No other factor was identified with an effect on PFS. Those patients responding to treatment with trastuzumab had a median PFS of 23.1 months (95% CI,12.4-36.6), while non-responders had a median PFS of 8.2 (95% CI,4.9-16.1) (p=0.001). In the group of patients with visceral metastases, median PFS was 11.1 months (95% CI,7.4-16.6), while in patients with no visceral involvement, this was 28 months (95% CI,14.9-46.1) (p=0.029).

Independent factors influencing progression-free survival by Cox regression analysis

Presence of visceral metastases and response to trastuzumab were identified as factors independently affecting PFS (Table 2).

The relation of PTEN and PI3K status and overall survival

Median OS was 26 months (95% CI,12.3-44.6) in the whole group of patients who received trastuzumab. OS was 25.1 months (95% CI,7.5-40.1) in PTEN expressing patients vs 26.8 months (95% CI,8.1-42) in non-expressing (p=0.5) (Figure 3A) and 24.8 months (95% CI,12.5-39.8) in the group of patients expressing PI3K vs 26.7 months (95% CI,9.8-43.2) in patients with no PI3K expression (p=0.42) (Figure 3B).

Factors influencing overall survival in univariate analysis

Response to trastuzumab and absence of visceral metastases were predictors of significantly prolonged OS. No other clinical or pathologic factors influenced OS. The median OS of responding patients was 32.2 months (95% CI,16.4-46.8), while non-responders lived a median of 24 months (95% CI,14.9-34.1). This difference was statistically significant (p=0.015).

In patients with visceral metastases, OS was 22.1 months (95% CI,17.2-32.3), while those patients with no visceral metastases enjoyed a median OS of 29.4 months (95% CI,15.6-42.3) (p=0.016)

Independent factors influencing overall survival by Cox regression analysis

Response to trastuzumab was identified as the single factor influencing survival by Cox regression analysis (Table 3).

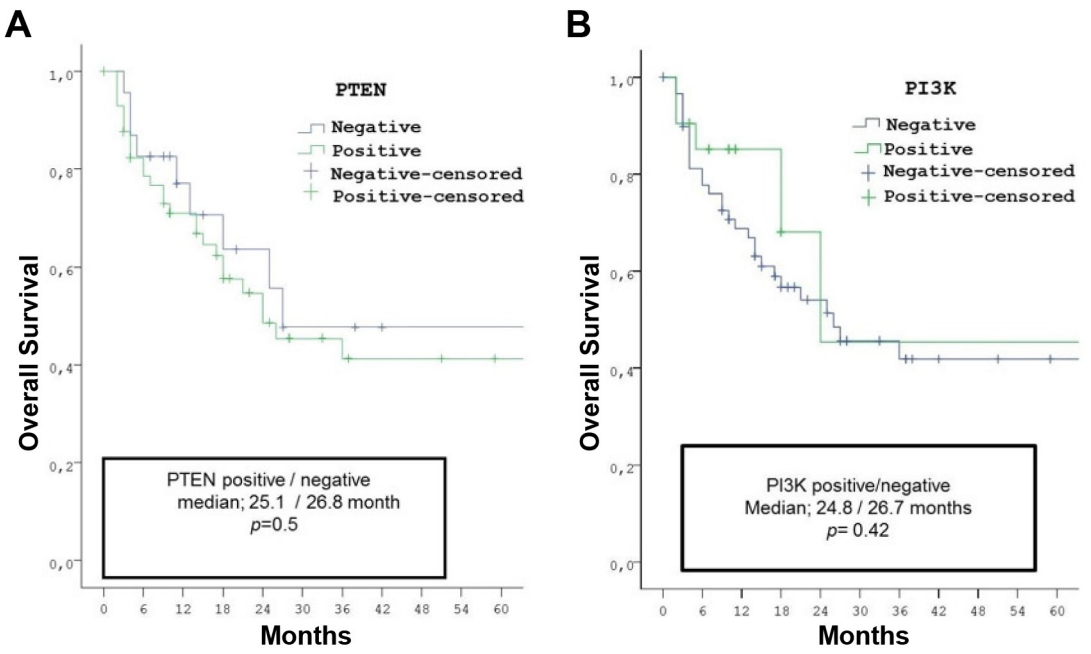

Figure 3. Patients' overall survival according to PTEN **(A)** and PI3K **(B)** statuses in HER2 positive metastatic breast cancer.

Table 3. Independent factors influencing OS by Cox regression analysis

Factors	*p*	*HR (95% CI)*
Trastuzumab treatment response	0.013	
Absent		1.7 (1.14-3.47)
Present		1

Discussion

Overexpression or amplification of HER2 is an established poor prognostic factor in breast cancer portending an aggressive course and potential for early metastasis. On the other hand, the monoclonal antibody trastuzumab is widely used in the clinic to target this overexpressed oncogene. Unfortunately, only approximately 30-40% of all patients overexpressing ERBB2 respond to trastuzumab, and this calls for further research regarding the additional modulation of the receptor. In this study, we aimed to investigate the response to trastuzumab in terms of the potential roles of several oncogenic pathways (PTEN and PI3K) by retrospectively analyzing paraffin-embedded archival material.

PTEN is a lipid phosphatase which acts as a 'tumor suppressor' by limiting the activity of the PI3K pathway, removing one phosphate from the catalytically active PIP3. Nagata et al. reported the findings from their *in vitro* studies that suggested trastuzumab inhibiting the PI3K/Akt pathway by activating PTEN. PTEN loss is hence associated with resistance to trastuzumab and might be a predictor for lower trastuzumab efficacy [8].

PTEN loss has been reported in 15 to 48% of breast cancer cases [10-13].

Wang et al. reported a shortened PFS associated with PTEN loss in a group of patients with metastatic breast cancer [13]. Esteva et al. showed that PTEN loss alone or in combination with PI3K mutations have predicted a decreased response to trastuzumab and a shortened survival [14]. Fabi et al. have reported that the PTEN-positive/p-Akt positive phenotype is associated with prolonged PFS but not with OS. However, no significant relationship between PTEN loss and trastuzumab-based treatment response was detected in several other studies in the neoadjuvant, adjuvant, and metastatic settings. In the study where Perez et al. have investigated the effect of PTEN in the three-arm adjuvant clinical trial of trastuzumab (chemotherapy alone, chemotherapy plus sequential trastuzumab, chemotherapy plus concurrent trastuzumab), no significant difference in disease-free survival was determined between PTEN positive and negative patients [15-17]. However, as these trials were done in the presence of chemotherapy, response to cytotoxic agents might interfere with the PTEN pathway and mask the pure HER2 resistance and its effect on response [18]. Additionally, in another trial investigating PTEN/PI3K pathway in neoadjuvant treatment with trastuzumab plus lapatinib without chemotherapy, PTEN loss or low PTEN expression levels were associated with trastuzumab resistance and therefore lower pathologic CR rates [19].

Staining intensity (strong, moderate, weak) and distribution has been the standard criteria for PTEN expression in previous studies [9]. Following Nagata's et al. study, IRS has replaced these criteria as the sole diagnostic criterion for PTEN expression [8]. We found a 43% PTEN loss in our study group by using IHC based on IRS scoring, which was consistent with what was previously reported in the literature. Our study also showed a lower rate of response to trastuzumab and shorter PFS for PTEN non-expressors (PFS 12.1 vs 15.3 months). PTEN expression was also associated with a significantly longer duration of trastuzumab efficacy, but no significant association with OS. This finding was also consistent with what was reported in the literature [8,13,14,20].

In retrospective studies, activating mutations of PI3K have been reported in 18 to 40% in breast cancer, the most common being point mutations involving exons 9 and 20. Exon 20 mutation H1047R is adjacent to the activation loop and gives rise to persistently elevated kinase activity [21-23]. PI3K mutations alone or in combination with PTEN loss are also reported to be associated with resistance to trastuzumab. In the study by Berns et al., PI3K mutations alone did not have a significant impact on PFS. However, in patients with both PTEN loss and PI3K mutations, PFS decreased significantly [24]. Rimawi et al. showed that PI3KCA mutations have a role in trastuzumab resistance and may predict pathological complete response in the neoadjuvant setting [19]. In a genetic profiling study, only PI3K pathway genes were found overexpressed in HER2 positive metastatic breast cancer patients who progressed within a year [25]. Furthermore, Diaz-Serrano et al. showed that PI3K genomic alterations have a negative effect on trastuzumab response and outcome in HER2 positive advanced gastric cancer patients as well as in breast cancer [26]. However, in another genetic profiling study, PIK3CA mutations determined in 45.4% of metastatic breast cancer patients and PI3KCA status had no effect on PFS [27].

In our study, we used an antibody directed against the p85 regulatory subunit of PI3K. PI3K expression was noted in 42% of the patients. We were not able to show a relation of PI3K mutations, neither with response to trastuzumab nor with PFS and OS.

In the study by Fabi et al. 46% of 73 patients with ERBB2-positive breast cancer expressed PI3K, which was consistent with our findings, but contrary to our study, they have reported a positive association of the PTEN-positive/PI3K-positive phenotype with prolonged PFS but not OS [9]. They reported that Cox regression analysis did not confirm this finding. The study also failed to show that the same phenotype predicts the response to trastuzumab. PI3K was detected with IHC in the Fabi et al. study and in our study. Since genetic mutations are fundamental in order to develop resistance to trastuzumab therapy regarding the PI3K pathway, it should be noted that IHC is inadequate for determining proteins' activity.

The absence of visceral metastases predictably turned out to be a factor with a significantly favorable impact on both PFS and OS.

Cox regression analysis supported the data that suggested a response to trastuzumab and absence of visceral metastasis are significant prognostic variables for PFS and can help predict disease-free interval; OS of 32.2 months in the trastuzumab-responding group and 29.4 months in the group with no visceral metastasis further substantiate this conclusion. Furthermore, Cox regression analysis confirmed that response to trastuzumab is the single independent factor for OS.

Significant prolongation of PFS and OS in the trastuzumab-responding patients confirms that this is an important predictive factor. We also found out rather predictably that patients with visceral metastases do poorly in terms of both PFS and OS.

We found that PTEN loss occurred in 43% of our study group and correlated with resistance to trastuzumab and shortened PFS. Our study also showed that immunohistochemical determination of PI3K expression did not predict neither response to trastuzumab nor PFS or OS.

Anti-PI3K pathway therapies such as alpelisib and their combination with anti-HER2 agents are investigated and these strategies may become the basis of the breast cancer treatment in the future [28]. Clearly, there are conflicting results with PTEN and its relationship with response to anti-HER2 therapies, nonetheless, its predictive role should be elucidated with large prospective studies.

Conflict of interests

The authors declare no conflict of interests.

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