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Evaluation of the association of PIK3CA mutations and PTEN loss with efficacy of trastuzumab therapy in metastatic breast cancer

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Abstract

Background: Trastuzumab (T) is effective in metastatic breast cancer (MBC) with HER2 overexpression and/or amplification, but resistance to T develops in a significant number of HER2-positive patients. The understanding of the mechanisms of resistance is critical to the care of these patients.

Patients and methods: Formalin-fixed paraffin-embedded tumor tissue samples were collected from 256 patients with T-treated MBC. Clinical information was collected retrospectively from the patients' medical records. Central review of HER2 status by fluorescent in situ hybridization (FISH) and/or immunohistochemistry (IHC) revealed that of the 227 eligible patients only 139 (61%) were truly HER2-positive. PTEN, ER, PgR, and Ki67 were evaluated by IHC, while PTEN status was evaluated by FISH as well. PIK3CA mutations were identified with single nucleotide polymorphism (SNP) genotyping.

Results: Median time to progression (TTP) was 14.4 months for the HER2-positive and 10.3 for the HER2-negative patients (log-rank, $p=0.22$). Survival from the initiation of T (survival_T) was 50.4 months for the HER2-positive and 35.3 for the HER2-negative subgroups ($p=0.006$). Higher risk of progression was associated with HER2-positive status and the presence of PIK3CA mutations ($p=0.014$). PTEN loss, as determined by IHC, was associated with lower survival_T in the whole population ($p=0.029$) and in the HER2-positive population ($p=0.017$).

PIK3CA mutations and/or PTEN loss status were evaluated together as a single parameter, to estimate the impact of activation of the PI3K/AKT molecular pathway, and it was significantly associated with both decreased TTP ($p=0.003$ in the total population, $p=0.004$ in HER2-positive patients) and survival (survival_T, $p=0.011$ in total, $p=0.006$ in HER2-positive).

Conclusions: In this trastuzumab-treated breast cancer population, PIK3CA activating mutations were associated with shorter TTP and PTEN loss with decreased survival. The activation of the PI3K/AKT pathway from either defect was associated with both TTP and survival, indicating the adverse effect of this pathway's status on trastuzumab efficacy.

Keywords: breast cancer, HER2, trastuzumab, predictive, PTEN, PIK3CA

Introduction

The cell surface growth factor receptor HER2 is over-expressed in 15-20% of breast tumors usually due to HER2 gene amplification. This tumor characteristic is associated with worse prognosis and increased rates of disease recurrence [1]. In the last decade the use of the humanized monoclonal antibody, trastuzumab, that selectively targets the extracellular domain of the HER2 receptor, has proved to significantly improve objective response, time to disease progression and overall survival in patients with metastatic HER2 over-expressing and/or amplified tumors [2]. Unfortunately, a significant number of patients with HER2 amplified metastatic disease never respond to trastuzumab treatment, while resistance to treatment develops in the majority of responding patients within the first year of treatment [3,4]. Notably, it is relatively easy to rule out patients that are unlikely to benefit from trastuzumab treatment by evaluating HER2 overexpression or HER2 amplification, while on the other hand beneficial predictive factors to further separate HER2 positive patients to those who will benefit from trastuzumab from those who will not, have not been integrated into clinical practice.

Understanding the mechanisms of primary or secondary resistance to trastuzumab treatment is critical to predicting trastuzumab efficacy. Activation of the phosphoinositide 3-kinase (PI3K)/AKT pathway has been reported to confer resistance to trastuzumab treatment in breast tumors [5]. Oncogenic mutations of PIK3CA and low expression or loss of the phosphatase and tensine homologue gene (PTEN), that antagonizes the action of PIK3CA, have both been described as factors that activate the PI3K/AKT pathway and contribute to trastuzumab resistance in breast tumors [6,7]. Investigation in cell lines and clinical studies have suggested the possible use of the PIK3CA and PTEN status as predictive factors of trastuzumab efficacy in breast cancer [7-11] and in one study PIK3CA mutations were found to be mutually exclusive with PTEN loss [12]. In the present study we analyzed samples from patients with presumed HER2-positive breast tumors that were treated with trastuzumab, after local evaluation for HER2 by IHC. We then evaluated HER2, PTEN and PIK3CA status as well as their correlation with patient outcome.

Patients, Materials and Methods

The medical records of all patients with metastatic breast cancer (MBC) treated with trastuzumab-based combinations, between December 1998 and January 2010, were retrospectively reviewed. Eligibility criteria for this study were a: histologically confirmed MBC, b: adequacy of clinical data on patient's history, demographics, tumor characteristics, treatment details (drug dosages, schedule of administration, serious toxicities), and clinical outcome, c: availability of adequate formalin-fixed paraffin-embedded (FFPE) tumor tissue for biological marker evaluation, and d: trastuzumab-based treatment for metastatic disease. The translational research protocol was approved 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". Waiver of consent was obtained from the Bioethics Committee for patients included in the study before 2005. All patients included in the study after 2005, provided written informed consent for the provision of biological material for future research studies, before receiving any treatment.

We evaluated clinical data and tumor samples from patients with MBC, and what was thought to be HER2-positive disease by locally performed immunohistochemistry (IHC), that were treated off protocol with trastuzumab, with or without chemotherapy. All tumor samples were re-examined centrally for HER2 status by fluorescent in situ hybridization (FISH), or by IHC when FISH could not be performed. Additionally, estrogen receptor (ER), progesterone receptor (PgR) and Ki67 status were examined centrally by IHC.

For the purpose of this study Ki67, PTEN and PIK3CA were evaluated using the methods outlined below and patient outcome was correlated with the biomarkers in accordance with the latest guidelines concerning tumor marker prognostic studies (REMARK) [13].

Tissue micro-arrays (TMAs)

FFPE tumor tissue samples were retrospectively collected from 256 patients with MBC treated with trastuzumab. Nineteen cases were excluded for inadequate FFPE tumor tissue and 10 for being treated with trastuzumab in the neoadjuvant setting, thus decreasing the number of eligible/evaluable patients to 227. Seventeen TMA blocks

were constructed using a manual tissue microarrayer (Beecher Instruments, Sun Prairie, WI). For the construction of the TMA blocks, 2 core samples (1.5 mm in diameter) were obtained from representative regions of each of the tumor blocks. All markers were assessed by FISH or IHC, in sections cut from the TMA blocks or in whole tissue sections from the original blocks.

Immunohistochemistry (IHC)

TMA sections or whole tissue sections (3 μm thick) were stained for vimentin (clone V9, Dako, Glostrup, Denmark), cytokeratin 8/18 (clone 5D3, Leica Biosystems, Newcastle, U.K.), ER (clone 6F11, Leica Biosystems), PgR (clone 1A6, Leica Biosystems), HER2 (A0482 polyclonal Ab, Dako), Ki67 (clone MIB-1, Dako), and PTEN (clone 6H2.1, Dako) as previously described [14-16]. The evaluation of all IHC sections was done by pathologists, experienced in breast cancer and blinded as to the patients' clinical characteristics and survival data.

Interpretation of the IHC results

The ER and PgR immunostaining was scored using the Histoscore method. Tumors were classified as ER- or PgR-positive if staining was present in 1% or more of tumor nuclei [17]. HER2 protein expression was scored according to the recent guideline recommendations (scores from 0 to 3+) [18]. PTEN protein expression (cytoplasmic, nuclear or both) was evaluated according to a staining intensity scale from 0 (negative, no staining) to 3 (intense staining). Tumors with PTEN scores of 0 or 1 were considered to have PTEN loss. For Ki67, the expression was defined as low (<14%) and high (\geq 14%) based on the percentage of stained/unstained nuclei from the tumor areas [19].

Fluorescence in situ hybridization (FISH)

TMA sections or whole tissue sections (5 μm thick) were used for FISH analysis using the ZytoLight® SPEC HER2/TOP2A/CEN17 triple color probe kit (ZytoVision, Bremerhaven, Germany) and the LSI PTEN/CEP10 Dual Color Probe (Abbott Molecular, Des Plaines, IL). For all probes, sequential (5 planes at 1.0 μm) digital images were captured using the Plan Apo VC x100/1.40 objective (Nikon, Japan) using specific filters for each probe. The resulting images were reconstructed using specially developed software for cytogenetics (XCyto-Gen, ALPHELYS,

Plaisir, France). Four carcinoma cell lines (MDA-MB-231, MDA-MB-175, MDA-MB-453 and SK-BR-3) from the Oracle HER2 Control Slide (Leica) with a known HER2 gene status were also used as a control of the FISH assays and analyzed for HER2, topoisomerase II alpha and PTEN genomic status.

FISH evaluation

For the evaluation of HER2 gene status, 20 non-overlapping nuclei from the invasive part of the tumor were selected randomly, according to morphological criteria using DAPI staining, and scored. The HER2 gene was considered amplified when the ratio of the respective gene probe/centromere probe was ≥ 2.2 [18] and deleted when the ratio was ≤ 0.75 . In cases at or near the cut-off (1.8-2.2 for amplifications and 0.6-0.9 for deletions), additional 20 to 40 nuclei were counted and the ratio was recalculated. In cases with a borderline ratio from 60 nuclei, an additional FISH assay was performed in a whole section. For the PTEN gene, sixty non-overlapping nuclei from the invasive part of the tumor were randomly selected and scored. Deletion was considered to occur when the ratio of the gene probe/centromere probe was < 0.80 [20].

Single nucleotide polymorphism (SNP) genotyping for PIK3CA mutations

DNA was extracted from 182 FFPE whole tissue sections or macrodissected tissue fragments containing $>70\%$ tumor cells, using a fully automated isolation method based on silica-coated magnetic beads (Versant Tissue Preparation Reagents, Siemens Healthcare Diagnostics, Tarrytown, NY) in combination with a liquid handling robot, as previously described [21]. Mutation testing for PIK3CA E542K and E545K (exon 9) and H1047R (exon 20) was accomplished with custom Taqman-MGB-SNP genotyping assays (duplex qRT-PCR for the detection of control DNA and mutant target in the same reaction). Samples were normalized at 50ng/10ul reaction and were run in duplicates under default conditions for Allelic Discrimination in an ABI7500 sequence detection system equipped with the SDS v1.4 software (Applied Biosystems). Sequencing validation was performed in selected cases (n=4 for each assay).

Statistical Analysis

Data on selected patient and tumor characteristics, previous and subsequent lines of treatment, disease progression events and survival were obtained from medical records. Data were entered into a central database, and follow-up information for all patients was updated in February 2010. All examined biomarkers are presented as frequency and corresponding percentages, while associations with HER2 status were examined using the chi-square test or Fisher's exact test where appropriate. The majority of patients received trastuzumab in the 1st line of chemotherapy for metastatic disease and thus time to progression (TTP) was defined as the time from trastuzumab initiation in the 1st line treatment (with or without concurrent chemo/hormonal therapy) to the date of documented disease progression. Survival was measured (a) from diagnosis of first metastasis in all patients (survivalM) or (b) from initiation of trastuzumab treatment in the patients receiving trastuzumab as a 1st line treatment to the date of death (survivalT). Patients alive were censored at the date of the last follow-up contact. Survival probabilities were estimated by the Kaplan-Meier method. For the univariate and multivariate analyses, Cox proportional hazards models were used. Model choice was performed using backward selection criteria with $p < 0.10$, including in the initial step clinical parameters such as: age, menopausal status, performance status, receptor status (ER/PgR), Ki67 expression, number of metastatic sites, and HER2 status. Univariate analyses were performed in the centrally identified HER2 groups and interaction tests for each marker and HER2 were performed. Multivariate analysis was performed in the total cohort and in the population of clinical interest, i.e. HER2-positive patients. All tests are two-sided at the $\alpha = 0.05$ level of significance. No adjustment for multiple comparisons was performed. Analyses were conducted using SPSS 15.0.

Results

Among the 256 registered patients, 227 were evaluated centrally for HER2 and 139 were found to have HER2 gene amplification by FISH and/or 3+ HER2 protein overexpression. We identified only one case with HER2 protein expression 3+ and non-amplified HER2 gene status. Eighty-eight patients had thus received trastuzumab-based therapy despite being HER2-negative, probably because of inexperience with HER2 IHC evaluation at the local laboratories in the early years of

trastuzumab use. Selected patient and tumor characteristics of both groups are seen in Table 1. Median follow-up for all patients was 66 months, while for patients treated with trastuzumab in 1st line, median follow-up was 51 months. 135 patients (59%) received more than one line of therapy with trastuzumab. 192 patients (85% of the 227 analyzed) were treated with trastuzumab in the 1st line of therapy for metastatic disease and of those 186 received concurrent chemo/hormonal therapy. Six patients received trastuzumab monotherapy, while the rest received trastuzumab in combination with chemotherapy agents, such as taxanes (61%), platinum (12%), gemcitabine (18%), and vinorelbine (11%). In the entire cohort, one patient had been treated with lapatinib before trastuzumab, whereas 35 received lapatinib after trastuzumab. Nine patients had received trastuzumab in the adjuvant setting as well, 6 of them relapsing within a year and 2 within six months. Only 2 of those patients' tumors carried a PIK3CA mutation. Among the 192 patients treated in 1st line, 151 (79%) progressed and 108 (56%) died. Median TTP was 13.7 months (95% Confidence Interval [CI] 10.9-16.5), while median survival_T was 42 months (95% CI 34.3-49.5). Of 227 patients analyzed, 138 (61%) died (median survival_M 47.5 months, 95% CI 40.9-54.1). HER2-positive patients had significantly longer survival than HER2-negative patients (median survival_M 39.1 months in HER2-negative vs 54.1 months in HER2-positive, $p=0.036$; median survival_T 35.3 in HER2-negative vs 50.4 months in HER2-positive, $p=0.006$), but TTP of those who received trastuzumab as 1st line treatment was not different between the two groups (10.3 months in HER2-negative vs 14.4 in HER2-positive, $p=0.22$). This is probably because in 1st line treatment a strong chemotherapy effect seen in both groups, dilutes any possible trastuzumab effect.

Association of markers with HER2 status

ER positivity was seen more frequently in HER2-negative patients (82% in HER2-negative vs. 59% in HER2-positive, $p=0.001$). PTEN loss by IHC was more common in HER2-negative patients (65% vs 50% in HER2-positive, $p=0.048$), as were PIK3CA mutations (30% vs 16% in HER2-positive, $p=0.039$) (Table 2). PIK3CA mutations were more common in ER-positive patients (27% in ER-positive vs 11% in ER-negative, $p=0.017$), while PTEN loss was more frequent in low Ki67 tumors (90% in low Ki67 vs 53% in high Ki67, $p=0.024$).

PTEN loss and PIK3CA mutation are thought to be mutually exclusive [22]. However, we identified 17 cases (among 153 tumors evaluated by both methods) where PIK3CA was mutated in the presence of PTEN loss, 10 of which were HER2-negative. PTEN status was also evaluated by FISH, and was not found to correlate with PTEN status by IHC.

Association of markers with clinical outcome

In the univariate analysis performed in the HER2-positive patients, PIK3CA mutations were associated with increased risk of progression (Hazard Ratio [HR]=2.50, 95% CI 1.35-4.61, Wald-p=0.003) (Figure 1), but not with survival (survivalT, Wald-p=0.27; survivalM, Wald-p=0.29). PTEN loss was associated with increased risk of death (HR=1.92, 95% CI 1.11-3.31, Wald-p=0.019; HR=1.73, 95% CI 1.04-2.89, Wald-p=0.035) (Figure 2), but not with TTP (Wald-p=0.16). Of note, PTEN status by FISH was not associated with TTP (Wald-p=0.66) or survival (Wald-p=0.51; Wald-p=0.91) in the univariate analysis. In the HER2-negative patients there was no significant association of PIK3CA mutations or PTEN loss with TTP, survivalT or survivalM. Tests for interaction of PIK3CA mutations or PTEN protein expression with HER2 status were not significant (Wald-p>0.05 for all survival parameters).

In the multivariate analysis performed in the entire population (Table 3), HER2-positive status was associated with a lower risk of disease progression (HR=0.60, 95% CI 0.38-0.95, Wald-p=0.028) and PIK3CA mutations with a higher risk of progression (HR=1.86, 95% CI 1.13-3.05, Wald-p=0.014). The association of risk of relapse with PTEN status did not reach statistical significance (HR=0.67, 95% CI 0.44-1.02, Wald-p=0.062). In terms of survival, PIK3CA mutation status was not associated with risk of death. However, PTEN loss was associated with a higher risk of death (HR=1.85, 95% CI 1.24-2.76, Wald-p=0.003 for survivalM; HR=1.64, 95% CI 1.05-2.57, Wald-p=0.029 for survivalT). Survival from the initiation of trastuzumab was associated with HER2 status (HER2-positive vs negative, HR=0.51, Wald-p=0.004) and adjuvant chemotherapy use (HR=1.64, Wald-p=0.038), whereas survival measured from either time point (survivalT vs survivalM) was associated with the number of metastatic sites (HR=1.71, Wald-p=0.029 and HR=1.56, Wald-p=0.036, respectively) and patients' performance status (HR=2.31 and HR=2.51, respectively, both Wald-p<0.001).

We also performed a multivariate analysis on the HER2-positive population, since this is ultimately the clinically relevant population (final model not shown). In this analysis, PIK3CA mutations were the only parameter significantly associated with worse TTP (HR=2.50, 95% CI 1.35-4.61, Wald-p=0.003). In terms of survival, poor performance status was associated with worse survivalM (HR=1.99, 95% CI 1.17-3.42, Wald-p=0.012). Increased number of metastatic sites at trastuzumab initiation was associated with worse survivalT (HR=2.20, 95% CI 1.15-4.23, Wald-p=0.017) but not significantly so with survivalM, while PTEN loss with shorter survival, as measured from either time point (HR=1.92, Wald-p=0.017, survivalT; HR=1.69, Wald-p=0.047, survivalM).

PIK3CA mutations and/or PTEN loss were also evaluated together, as a single parameter, in the multivariate analysis, to estimate the impact of the activation of the molecular pathway. In the HER2-positive population, it was found to be significantly associated with both worse TTP (HR=2.16, 95% CI 1.27-3.66, Wald-p=0.004) and survival (HR=2.12, Wald-p=0.041, survivalT; HR=2.51, Wald-p=0.006, survivalM) (Table 4). Kaplan-Meier curves for PIK3CA mutations and/or PTEN loss, as one variable, in terms of TTP and survivalT are given in Figure 3. Results were similar in the whole population.

Discussion

Our study is among the first to evaluate both PIK3CA mutations and PTEN protein expression status in trastuzumab-treated patients, and it confirms the results of previous investigators that these two markers are involved in the development of resistance to trastuzumab [5-12,23,24].

PTEN loss was associated with shorter survival, whether measured from first development of metastasis or from trastuzumab initiation, both in the entire population and, more importantly, in HER2-positive patients. Additionally, there is a trend for longer TTP in total population without PTEN loss that doesn't quite reach statistical significance. These findings are consistent with the prognostic role of PTEN in HER2-positive, trastuzumab-treated patients. On the other hand the effect of PIK3CA activating mutations was mostly an effect on the immediate action of trastuzumab and was thus seen as a prolongation of TTP. Additionally, PIK3CA

mutations appear to correlate with good prognostic indicators, such as ER and PgR expression [12,20]. Another interesting finding is the difference in the “areas of influence” of PI3K and PTEN. Why should PI3K, though it is a critical molecule in the AKT pathway, influence TTP more than survival and PTEN the reverse, is something that needs to be explored further. Could very small, statistically insignificant, incremental benefits in TTP, in several lines of treatment with trastuzumab, lead to a cumulative survival gain?

Clearly, what is most relevant is the activation of the entire pathway. Hence the combined analysis of both markers yielded a strong association with both survival and TTP, thus confirming the significance of this pathway in trastuzumab resistance.

A recent study evaluated the clinical significance of PTEN and pAKT in HER2-positive disease and found that when both PTEN and pAKT are overexpressed patients enjoy longer progression-free survival (PFS) on trastuzumab [9]. In this prospective 1st line trastuzumab/chemotherapy study, PI3K was also evaluated by IHC, but its overexpression did not seem to be associated with PFS. The authors, however, note that IHC is probably not the optimal way to assess PI3K activity and that PIK3CA mutations are much more relevant in this setting [9]. PTEN status on the other hand is best evaluated by IHC.

Additional interest in our study derives from the evaluation of the HER2-negative, trastuzumab-treated patients. In this subgroup of patients, survival from the initiation of trastuzumab and from first diagnosis of metastatic disease was shorter than in the HER2-positive patients. This finding underlines the dramatic effect of trastuzumab on the survival of HER2-positive patients, who without trastuzumab, would be expected to have more aggressive disease and worse prognosis [1].

In HER2-negative patients the effect of treatment was not influenced by the presence of PIK3CA mutations and/or PTEN loss. It is particularly interesting to examine this HER2-negative subgroup, since it is unlikely that one will ever come across such a population in the future, as HER2 testing methods have improved and cost regulation and established treatment guidelines would prevent the use of trastuzumab in HER2-negative patients. However, one could have postulated that in the setting of wild type PIK3CA or PTEN overexpression, trastuzumab could have exerted a therapeutic effect in a “HER2-borderline” population, or even that trastuzumab could have an effect that would possibly be unrelated to HER2 and more dependent on downstream molecules. There was a recent review suggesting that, as HER2 positivity is more

significant in stem cells, in the adjuvant setting, trastuzumab may be active in HER2 1+ or 2+ cases, as well [25]. Our results however, render such hypotheses unlikely, at least in the metastatic setting, and reinforce the importance of HER2 overexpression or amplification in predicting benefit from trastuzumab.

Our study is fraught with the disadvantages of all retrospective analyses, especially those that come from a non-trial population. Our results should thus be considered as hypothesis generating, while results of prospective studies, such as the NEO-ALTO and ALTO studies, are eagerly awaited to clarify the field. Meanwhile the scientific and clinical community has to establish optimal ways to evaluate PI3K and PTEN expression and standardize the methods and cut-offs.

In conclusion, the efficacy of trastuzumab therapy in patients with HER2-positive MBC is highly dependent on the activation of the PI3K/AKT pathway, induced by activating PIK3CA mutations or PTEN loss. Further studies are necessary to validate the predictive value of PIK3CA mutations and PTEN loss especially in light of the new therapies targeting the PI3K/AKT pathway.

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References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235 (4785):177-182
2. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344 (11):783-792. doi:10.1056/NEJM200103153441101
3. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 20 (3):719-726
4. Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Anton A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 23 (19):4265-4274. doi:JCO.2005.04.173 [pii] 10.1200/JCO.2005.04.173
5. Nahta R, Yu D, Hung MC, Hortobagyi GN, Esteva FJ (2006) Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract Oncol* 3 (5):269-280. doi:ncponc0509 [pii] 10.1038/ncponc0509
6. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12 (4):395-402. doi:S1535-6108(07)00262-0 [pii] 10.1016/j.ccr.2007.08.030
7. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 6 (2):117-127. doi:10.1016/j.ccr.2004.06.022 S1535610804002107 [pii]
8. Fujita T, Doihara H, Kawasaki K, Takabatake D, Takahashi H, Washio K, Tsukuda K, Ogasawara Y, Shimizu N (2006) PTEN activity could be a predictive marker of trastuzumab efficacy in the treatment of ErbB2-overexpressing breast cancer. *Br J Cancer* 94 (2):247-252. doi:6602926 [pii] 10.1038/sj.bjc.6602926
9. Fabi A, Metro G, Di Benedetto A, Nistico C, Vici P, Melucci E, Antoniani B, Perracchio L, Sperduti I, Milella M, Cognetti F, Mottolise M (2010) Clinical significance of PTEN and p-Akt co-expression in HER2-positive metastatic breast cancer patients treated with trastuzumab-based therapies. *Oncology* 78 (2):141-149. doi:000312656 [pii] 10.1159/000312656
10. Gori S, Sidoni A, Colozza M, Ferri I, Mameli MG, Fenocchio D, Stocchi L, Foglietta J, Ludovini V, Minenza E, De Angelis V, Crino L (2009) EGFR, pMAPK, pAkt and PTEN status by immunohistochemistry: correlation with clinical outcome in HER2-positive metastatic breast cancer patients treated with trastuzumab. *Ann Oncol* 20 (4):648-654. doi:mdn681 [pii] 10.1093/annonc/mdn681
11. Pandolfi PP (2004) Breast cancer--loss of PTEN predicts resistance to treatment. *N Engl J Med* 351 (22):2337-2338. doi:351/22/2337 [pii]

10.1056/NEJMcibr043143

12. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* 65 (7):2554-2559. doi:65/7/2554 [pii]

10.1158/0008-5472-CAN-04-3913

13. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2006) REporting recommendations for tumor MARKer prognostic studies (REMARK). *Breast Cancer Res Treat* 100 (2):229-235. doi:10.1007/s10549-006-9242-8

14. Fountzilas G, Bobos M, Kalogera-Fountzila A, Xiros N, Murray S, Linardou H, Karayannopoulou G, Koutras AK, Bafaloukos D, Samantas E, Christodoulou C, Economopoulos T, Kalogeras KT, Kosmidis P (2008) Gemcitabine combined with gefitinib in patients with inoperable or metastatic pancreatic cancer: a phase II Study of the Hellenic Cooperative Oncology Group with biomarker evaluation. *Cancer Invest* 26 (8):784-793. doi:902506207 [pii]

10.1080/07357900801918611

15. Gogas H, Pectasides D, Kostopoulos I, Lianos E, Skarlos D, Papaxoinis G, Bobos M, Kalofonos HP, Petraki K, Pavlakis K, Bafaloukos D, Fountzilas G (2010) Paclitaxel and carboplatin as neoadjuvant chemotherapy in patients with locally advanced breast cancer: A phase II Trial of the Hellenic Cooperative Oncology Group. *Clin Breast Cancer* 10 (3):230-237. doi:4890M3333Q3781R5 [pii]

10.3816/CBC.2010.n.031

16. Bamias A, Karina M, Papakostas P, Kostopoulos I, Bobos M, Vourli G, Samantas E, Christodoulou C, Pentheroudakis G, Pectasides D, Dimopoulos MA, Fountzilas G (2010) A randomized phase III study of adjuvant platinum/docetaxel chemotherapy with or without radiation therapy in patients with gastric cancer. *Cancer Chemother Pharmacol* 65 (6):1009-1021. doi:10.1007/s00280-010-1256-6

17. Hugh J, Hanson J, Cheang MC, Nielsen TO, Perou CM, Dumontet C, Reed J, Krajewska M, Treilleux I, Rupin M, Magherini E, Mackey J, Martin M, Vogel C (2009) Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an immunohistochemical definition in the BCIRG 001 trial. *J Clin Oncol* 27 (8):1168-1176. doi:JCO.2008.18.1024 [pii]

10.1200/JCO.2008.18.1024

18. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 25 (1):118-145. doi:JCO.2006.09.2775 [pii]

10.1200/JCO.2006.09.2775

19. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, Perou CM, Ellis MJ, Nielsen TO (2009) Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 101 (10):736-750. doi:djp082 [pii]

10.1093/jnci/djp082

20. Christodoulou C, Kostopoulos I, Kalofonos HP, Lianos E, Bobos M, Briasoulis E, Gogas H, Razis E, Skarlos DV, Fountzilas G (2009) Trastuzumab combined with pegylated liposomal doxorubicin in patients with metastatic breast cancer. phase II Study of the Hellenic Cooperative Oncology Group (HeCOG) with biomarker evaluation. *Oncology* 76 (4):275-285. doi:000207504 [pii]

10.1159/000207504

21. Bohmann K, Hennig G, Rogel U, Poremba C, Mueller BM, Fritz P, Stoerckel S, Schaefer KL (2009) RNA extraction from archival formalin-fixed paraffin-embedded tissue: a comparison of manual, semiautomated, and fully automated purification methods. *Clin Chem* 55 (9):1719-1727. doi:clinchem.2008.122572 [pii]

10.1373/clinchem.2008.122572

22. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors,

Table 1. Selected patient and tumor characteristics (at trastuzumab initiation) according to HER2 status (*HER2-positive; HER2-amplified and/or HER2 IHC 3+*)

	HER2 status	
	Negative	Positive
N	88	139
Age (years) ¹		
Median (range)	59 (32-79)	54 (28-95)
	N (%)	N (%)
Menopausal status		
Premenopausal	26 (29.5)	43 (30.9)
Postmenopausal	62 (70.5)	96 (69.1)
Performance status		
0	56 (63.6)	97 (69.8)
1	18 (20.5)	28 (20.1)
2	8 (9.1)	6 (4.3)
Unknown	6 (6.8)	8 (5.8)
History of adjuvant CT	55 (62.5)	77 (55.4)
Anthracycline-containing CT	30 (33.7)	61 (43.9)
Taxane-containing CT	17 (19.3)	38 (27.3)
CMF-like CT	29 (33.0)	44 (31.7)
History of adjuvant HT	42 (47.7)	62 (44.6)
History of adjuvant RT	33 (37.5)	53 (38.1)
Grade (at initial diagnosis)		
1	2 (2.3)	4 (2.9)
2	36 (40.9)	49 (35.3)
3	42 (47.7)	76 (54.7)
Unknown	8 (9.1)	10 (7.2)
Sites of metastasis		
Locoregional	28 (31.8)	45 (32.4)
Distant	78 (88.6)	119 (85.6)
Only locoregional	5 (5.7)	10 (7.2)
Only distant	55 (62.5)	84 (60.4)
Bones	37 (42.0)	56 (40.3)
Visceral	58 (65.9)	95 (68.3)
Number of metastatic sites		
1	28 (31.8)	54 (38.8)
2	30 (34.1)	42 (30.2)
≥3	25 (28.4)	38 (27.3)
Unknown	5 (5.7)	5 (3.6)
Number of trastuzumab treatments		
1	39 (44.3)	53 (38.1)
2	19 (21.6)	33 (23.7)
3	12 (13.6)	23 (16.5)
≥4	18 (20.5)	30 (21.6)

node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer research 65 (7):2554-2559. doi:10.1158/0008-5472.CAN-04-3913

23. Perez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, Skoog L, Stal O (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 13 (12):3577-3584. doi:13/12/3577 [pii] 10.1158/1078-0432.CCR-06-1609

24. Pohlmann PR, Mayer IA, Mernaugh R (2009) Resistance to Trastuzumab in Breast Cancer. Clin Cancer Res 15 (24):7479-7491. doi:15/24/7479 [pii] 10.1158/1078-0432.CCR-09-0636

25. Kakarala M, Wicha MS (2008) Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. J Clin Oncol 26 (17):2813-2820. doi:26/17/2813 [pii] 10.1200/JCO.2008.16.3931

¹p=0,018; CT, chemotherapy; HT, hormonal therapy; RT, radiation therapy

Table 2. Association of examined markers with HER2 status

		HER2 status				Fisher's exact p
		Negative		Positive		
		N	%	N	%	
IHC						
ER (n=214)	Negative	15	17.9	53	40.8	0.001
	Positive	69	82.1	77	59.2	
PgR (n=212)	Negative	29	35.4	82	63.1	<0.001
	Positive	53	64.6	48	36.9	
Ki67 (n=203)	Low (<14%)	10	12.7	10	8.1	0.34
	High (≥14%)	69	87.3	114	91.9	
PTEN (n=182)	Loss (intensity 0-1)	46	64.8	55	49.5	0.048
	No loss (intensity 2-3)	25	35.2	56	50.5	
FISH						
PTEN (n=199)	Deletion (<0.80)	30	40.5	51	40.8	0.99
	Non deletion (≥0.80)	44	59.5	74	59.2	
SNP genotyping						
PIK3CA (n=175)	WT	49	70.0	88	83.8	0.039
	Mutated	21	30.0	17	16.2	

Table 3. Multivariate Cox regression analysis in the whole population treated with trastuzumab (T)

	HR	TTP 95% CI	Wald-p	HR	SurvivalT 95% CI	Wald-p	HR	SurvivalM 95% CI
	1			1			1	
	0.60	0.38-0.95	0.028	0.51	0.32-0.80	0.004	0.73	0.49-1.0
	1							
	0.67	0.42-1.06	0.086					
	0.98	0.97-1.00	0.084					
status at T initiation								
				1			1	
				2.32	1.47-3.65	<0.001	2.51	1.66-3.7
static sites at T								
				1			1	
				1.71	1.06-2.78	0.029	1.56	1.03-2.3
				1			1	
				1.64	1.03-2.61	0.038		
	1							
	1.86	1.13-3.05	0.014					
	1			1			1	
	0.67	0.44-1.02	0.062	0.61	0.39-0.95	0.029	0.54	0.36-0.8

, chemotherapy; SurvivalT and SurvivalM, See text

Table 4. Multivariate Cox regression analysis in the HER2-positive population

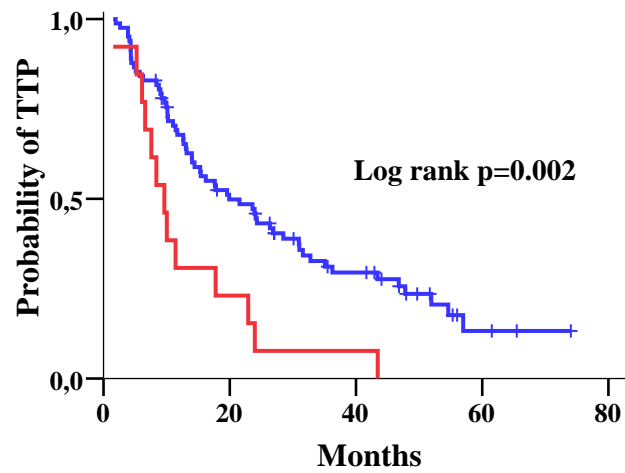
	TTP			SurvivalT		
	HR	95% CI	Wald-p	HR	95% CI	Wald-p
Age						
Performance status at T initiation						
0				1		
1-2				2.18	1.20-3.95	0.011
Number of metastatic sites at T initiation						
<3				1		
≥3				1.95	1.03-3.70	0.041
PIK3CA/PTEN co-expression						
Wild type PIK3CA and PTEN no loss (2-3)	1			1		
Mutated PIK3CA and/or PTEN loss (0-1)	2.16	1.27-3.65	0.004	2.51	1.31-4.82	0.006

Note: When the PTEN and PIK3CA parameters were entered separately in the analysis, PIK3CA mutations was the only parameter significantly associated with worse TTP (HR=2.50, 95% CI 1.35-4.61, p=0.003), while PTEN loss was associated with shorter survival as measured from either time point (HR=1.92, p=0.017, survivalT; HR=1.69, p=0.047, survivalM).

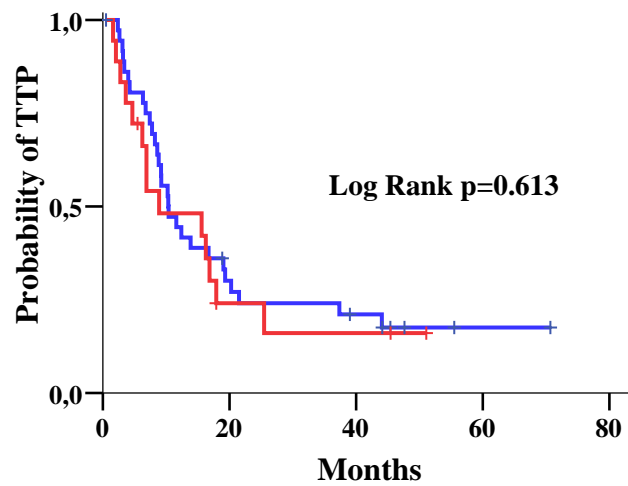
T, trastuzumab; SurvivalT and SurvivalM, See text

Figure 1. Kaplan-Meier curves for TTP according to PIK3CA gene status.

A. HER2-positive subgroup



B. HER2-negative subgroup

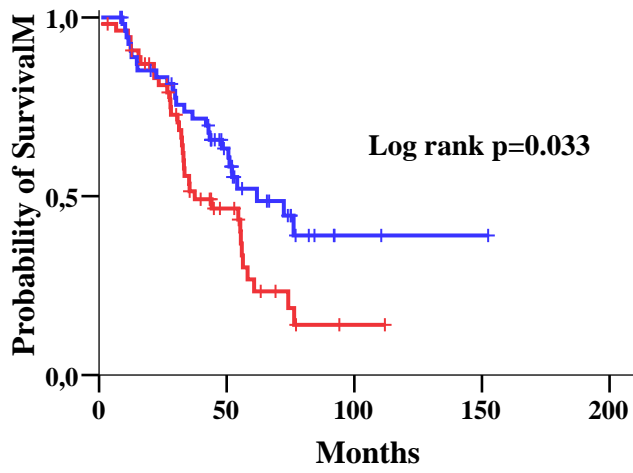


— Wild type (WT)

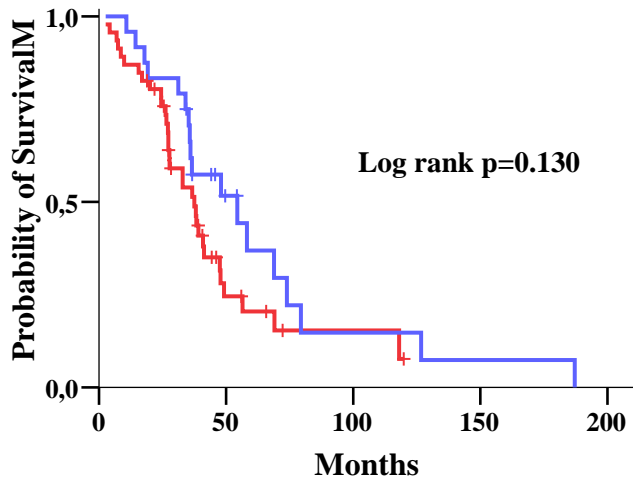
— Mutated

Figure 2. Kaplan-Meier curves for survivalM according to PTEN protein expression

A. HER2-positive subgroup



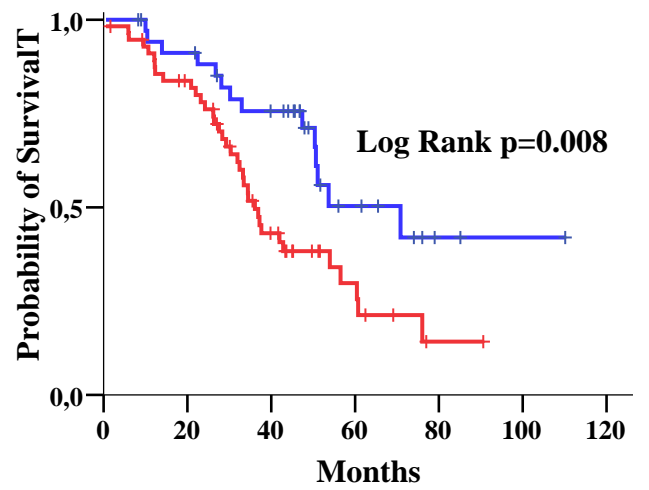
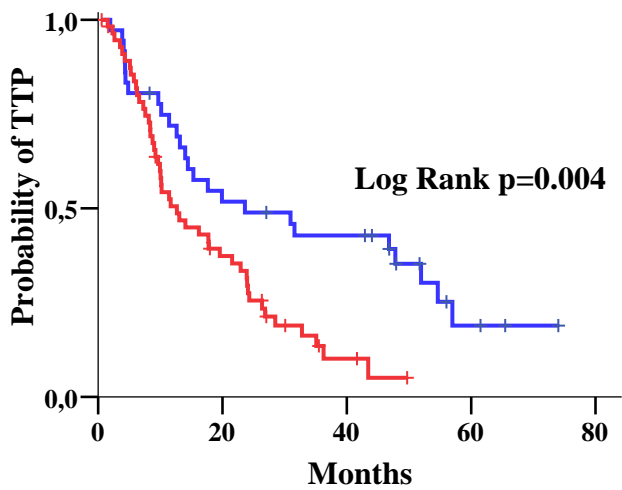
B. HER2-negative subgroup



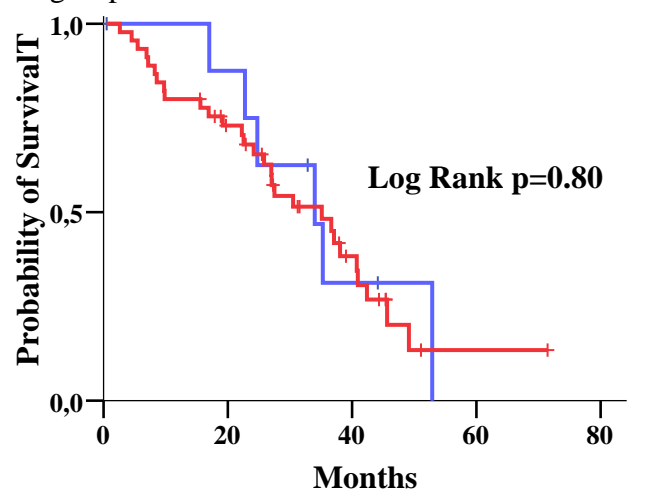
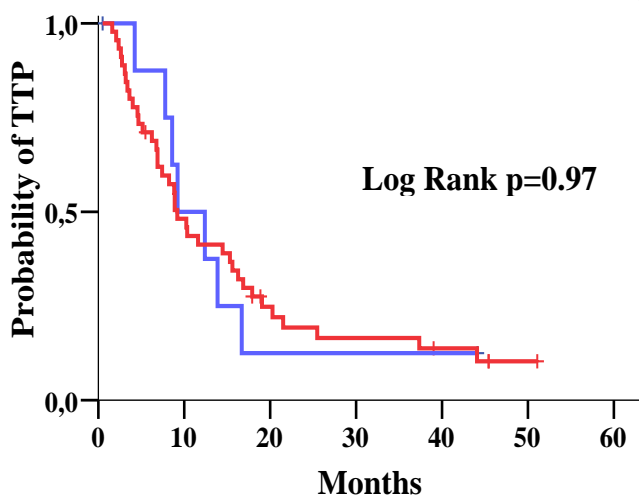
— No loss

— Loss

Figure 3. Kaplan-Meier PTEN/PIK3CA co-evaluation for TTP and survivalT
A. HER2-positive subgroup



B. HER2-negative subgroup



- PIK3CA wt and PTEN no loss
- PIK3CA mutations and/or PTEN loss

BREAST CANCER RESEARCH AND TREATMENT

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